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***Effect of organic nutrient perturbation on
microbial community dynamics and
composition in Scottish coastal waters***

Romain Pete

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Abstract

This study investigates the effect of organic nutrient perturbation on the dynamics of marine microbial communities, adopting three different approaches. A field study was conducted on the west coast of Scotland, in two contrasting locations, including a fish farm anticipated as a point source of organic nutrients. Data collected from the stations sampled revealed that the marine microbial communities were highly dynamic. The comparison of the physico-chemical and biological variables measured at each station indicated that the abundances, diversities and productions of planktonic micro-organisms varied in time and space. These marine microbes also demonstrated strong ecological interactions such as competition or predation. The dissolved organic matter (DOM) was revealed to originate from various sources with production by phytoplankton being dominant. Laboratory based experiments were conducted to complement field observations and test different hypotheses, such as the effect of substrate organic stoichiometry on the growth of bacteria and their grazers, the effect of DOM produced by nutrient-stressed phytoplankton on the bacterioplankton dynamics or the factors limiting the natural microbial communities. These experiments highlighted the key role of bacteria within the microbial loop, efficiently regenerating DOM into inorganic nutrients, the importance of the quality and stoichiometry of the DOM in controlling bacterial growth. Additionally in these experiments, the manipulation of size-fractionated microbial food webs revealed the importance of micro-grazers in controlling the bacterioplankton dynamics. Finally, mathematical modelling was used to synthesise the field and experimental observations and to simulate ecological interactions concurrent to organic nutrient variations. An existing model was modified to include the bacterioplankton component as well as a more adequate DOM pool representation.

A mes parents, Alain et Françoise

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- CHAPTER 1 -

General introduction

1.1 Marine microbial food web

1.1.1 Microbial ecology

Microbial ecology aims to study the interactions between organisms and with their surrounding environment. In the marine environment, microbial food webs are complex, dynamic networks of specifically and functionally interacting organisms. The evidence of the key role played by marine microbes, such as marine protists, heterotrophic bacteria and cyanobacteria, is now well supported by numerous studies. These microbes are a critical link in the “microbial loop”. This microbial loop starts with the production of dissolved organic matter (DOM) and, through heterotrophic bacteria and the processes they mediate, ends with more respiration, nutrient cycling, growth of bacteria and their grazers.

1.1.2 Diversity amongst microbial organisms

Marine microbes are single celled organisms grouped in three divisions of life: Bacteria, Archea and Eukarya (Fig. 1.01), based on the study of subunits (16S and 18S) of ribosomal ribonucleic acid (rRNA) (Woese et al. 1990). These microbes exhibit a wide range of cell lengths, this being used as a method of classification (Table 1.01) by Seiburth et al. (1978). These organisms comprise both prokaryotic (no nuclear membrane) and eukaryotic (distinct nucleus) cells and cover a wide range of trophic

regimes from photoautotrophy (organisms using light as the source of energy) to heterotrophy (living on organic matter). Marine microbes also use a variety of energy sources, elements for biosynthesis and reducing equivalent (redox pairs e.g. $\text{H}_2\text{O}/\text{O}_2$, NADPH/NADP , HS^- , NO_3^-).

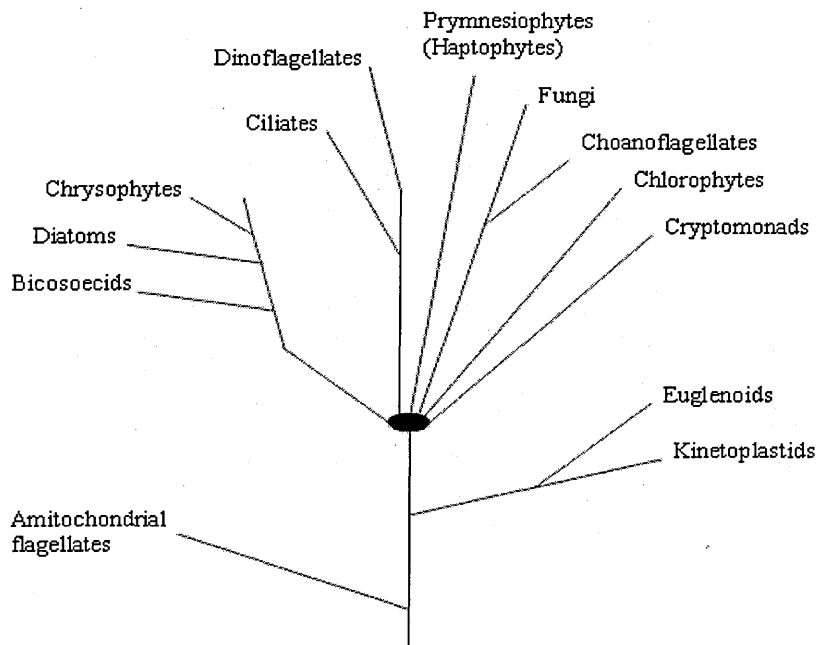


Figure 1.01. Phylogenetic tree of eukaryotic microbes. Relationship based on SSU rRNA (from Rappé et al. 2000).

1.1.3 From the Classical view to the modern paradigm

The classical view of the marine pelagic food chain, i.e. inorganic nutrients taken up by phytoplankton which are in turn ingested by zooplankton which are ingested by fish (Steele 1970, Steele 1974), has changed significantly within the last three decades because of an appreciation of new “actors” in marine food webs. In his review, Fenchel (1988) drew a new scheme of marine plankton food chain (Fig. 1.02) by introducing the, so-called, microbial loop (Pomeroy 1974, Azam et al. 1983, Murray & Eldridge 1994).

Although bacteria were already suggested as decomposers, the new picture of plankton based on this microbial loop brought a “change in paradigm” by recognising that phototrophic and heterotrophic microorganisms (in particular bacteria and microflagellate grazers) play a substantial or even sometimes dominating role in the cycling of matter in the sea (LeB Williams 1981, Azam et al. 1983).

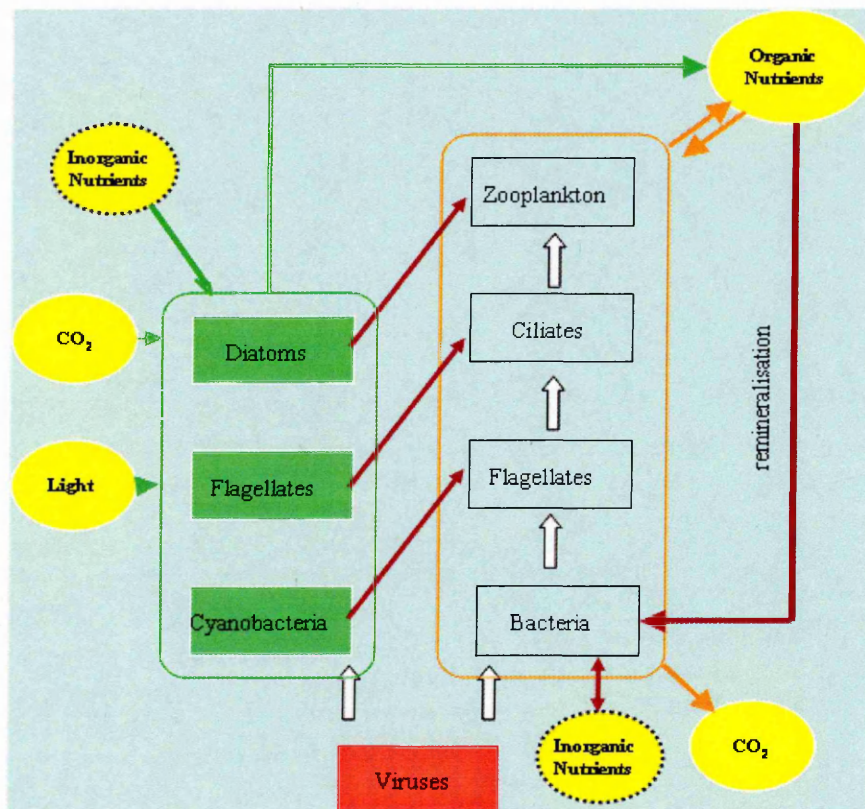


Figure 1.02. Conceptual diagram of the microbial food web with autotrophic organisms (green frame) and heterotrophic organism (orange frame). Green arrows represents autotrophic processes of inorganic nutrient uptake and DOM production by phytoplankton. Brown arrows represent heterotrophic processes with consumption of DOM by bacteria, remineralisation into inorganic nutrient, and grazing of phytoplankton. White arrows represent the existing trophic links between heterotrophic organisms (redrawn from Fenchel, 1988).

Bacteria can, under this new paradigm, both enter into competition with phytoplankton for inorganic resources and also act as a major pathway of organic matter decomposition, both particulate and dissolved (Dugdale & Goering 1967, Ducklow 1983, Andersen & Fenchel 1985, Biddanda 1985, Caron et al. 1985, Goldman et al.

1985, Alldredge et al. 1986, Andersen & Sorensen 1986, Biddanda 1986, Ducklow et al. 1986, Biddanda 1988, Biddanda & Pomeroy 1988).

Nowadays, we know that, in most of the ocean, organic matter passes through the microbial loop. An average of 50 % of the primary production (PP) is consumed by bacteria, as photosynthetic exudates, the contents of dead-cells (released by bacterial and viral lyses, zooplankton sloppy-feeding) or zooplankton excretions (Fuhrman & Azam 1982, Azam et al. 1983, Ducklow et al. 1986, Azam 1998).

Table 1.01. General groups of pelagic microbes in the sea

Size category	Microbial group	Size range (µm)
Femtoplankton	Viruses	0.01-0.2
Picoplankton	Prokaryotes	
	Bacteria	
	Phototrophic	0.5-1.0
	Prochlorophytes	0.5-2.0
	Cocoids cyanobacteria	0.5-1.0
	Filamentous cyanobacteria	7-10 wide x ≤100s long
	Chemoautrophic	0.3-1.0
	Heterotrophic	0.3-1.0
	Archea	
	Eukaryotes	
Nanoplankton	Picoalgae, picoheterotrophic flagellates	1.0-2.0
	Nanoalgae, nanoheterotrophic protists (mainly flagellates)	2-20
Microplankton	Microalgae	
	Microheterotrophic protists (mainly ciliates and heterotrophic dinoflagellates)	20-200

Microbial size categories proposed by Sieburth (1978), after Sherr et al. (2000).

In addition to bacteria, heterotrophic micro-flagellates are important in the microbial loop. Microflagellates, which graze on both phytoplankton and heterotrophic bacterioplankton, play a major role in marine food webs by removing the organic matter from the linear grazing model phytoplankton-zooplankton-fish (LeB Williams 1981, Estep et al. 1986, Fenchel 1986). Furthermore, these micrograzers are able to convert

organic matter into inorganic nutrients, hence enabling further production (LeB Williams 1981).

1.2 Marine heterotrophic bacteria

1.2.1 Diversity and molecular ecology

Studies in the early nineties (Giovannoni et al. 1990) that used rRNA small subunit (SSU rRNA) revealed the existence of several bacterial taxa, uncultured or unknown until then, amongst the prokaryote kingdom. SSU rRNA genes sequence comparison demonstrated that most (80 %) bacteria fall into nine phylogenetic groups (Giovannoni & Rappe 2000). Most of these marine bacteria fall into the γ subclass of the *proteobacteria*, with lower numbers comprising in the *Cytophaga-Flavobacterium-Bacteriodes* group and the α subclass of *proteobacteria*. Numerous studies have demonstrated a range of abilities of these different subclasses of marine bacteria. For example, the SAR 11 clade, belonging to the α -*proteobacteria*, seems to dominate the upper layer of oligotrophic ocean (Field et al. 1997). The β -*proteobacteria* subgroup, however, is reported to be located in coastal waters (Rappé et al. 1997). Moreover, there is molecular evidence of great diversity among the heterotrophic prokaryotes, only a very few (less than 1%) have been isolated and grown in cultures (Rappé & Giovannoni 2003). With the vast majority of bacteria being in the uncultured fraction, one may expect that diversity and function will be found to increase even further.

1.2.2 Role of bacteria in marine systems

Marine heterotrophic bacteria are the largest surface area of living organisms in the ocean (LeB Williams 1981). Because of their diversity and their key role in the processes they mediate in the oceans, marine heterotrophic bacteria have been of fast growing interest to marine microbial ecologists. Heterotrophic bacteria are closely related to DOM dynamics and they exhibit a wide range of interactions with the DOM pool (see section 1.3.2.4, this Chapter). Marine bacteria therefore appear to be a key player in terms of carbon (C) budget. Bacteria remineralise this DOM into inorganic form, hence potentially promoting further primary production and hence more OM (see section 1.3.2.4, this Chapter) and may paradoxaly enter in competition for these inorganic nutrients with the provider of their source of energy: phytoplankton (Bratbak & Thingstad 1985, Thingstad 1987). Therefore, it remains unclear whether or not marine heterotrophic bacteria are a net sink or source of C in the ocean (see section 1.3.2.4, this Chapter and also Chapter 6). Clearly, they interact with other organisms, playing a central key role in microbial food webs, as noted above.

1.3 Nutrients in natural ecosystems, stoichiometry and nutrient transfer through the food chain

1.3.1 Inorganic nutrients

1.3.1.1 Composition and concentration

Seawater is composed of a number of major and minor inorganic (non-living) elements. Major elements (concentration > 1 mg/l) include the cations (Ca^{2+} , K^+ , Na^+) and elemental oxyanions: (Cl^- , SO_4^{2-} , HCO_3^-). Minor nutrients (concentration < 1mg/l) include vitamins (e.g. vitamin B12), trace metals (including Fe, Mg, Zn, Cu), and

nitrogen, phosphorous and silicon (as nitrate NO_3^- , nitrite NO_2^- , Ammonium NH_4^+ , silicate Si(OH)_4 and orthophosphate ions PO_4^{3-}). The typical concentration of trace metals is $\sim 10^{-8}$ μM and can also be limiting e.g. in high nutrient low chlorophyll (HNLC) regions. While minor elements, N, P and Si are (at least in coastal waters) the most important in seawater because one or other of these is most likely to be exhausted and hence limit primary production. The order of magnitude of the concentrations of nutrient concentration is about 10^{-6} M (Table 1.02).

Typically, nitrate, silicate and phosphate increase with depth. Occasionally marked changes occur with depth corresponding to the thermocline or chlorophyll maximum. Generally, the stratified surface layer is nutrient depleted because autotrophic organisms consume nutrients. Ammonium depth profiles show an opposite pattern with a decreasing concentration with depth. On an annual basis, nitrate and silicate concentrations are high during the winter and decrease as primary producers consume them during the spring bloom. Autumn and winter mixing supplied the surface waters with deep nutrient-enriched waters following breakdown of stratification. On the other hand, ammonium is released after nitrate consumption, mediated by heterotrophic remineralisation and this NH_4^+ is subsequently used to support a regenerated production during the summertime.

1.3.1.2 Nutrient ratio

Redfield (1963) demonstrated that elemental components are in balanced quantity in living marine organisms and defined the so called “Redfield ratio” of C:N:P to be 106:16:1. These molar ratios were reviewed by Brzezinski (1985) who added silicate and, while noting significant variability, suggested a C:N:P:Si ratio of 106:16:1:16. When phytoplanktonic cells are in balanced growth their ratios are

approximately those of Redfield but might respond differently under stressful conditions where nutrient concentrations change.

Phytoplankton cells in particular may exhibit large differences from this balanced C:N:P:Si ratio. As cells may continue to photosynthesise when N is limiting, it is common for cells to exhibit C:N ratios higher than Redfield. On the other hand lower than Redfield values are exhibited when light is limiting (Goldman et al. 1979, Falkowski et al. 1985, Smith et al. 1992b).

Table 1.02. Range of dissolved inorganic and organic nutrient concentrations in marine environments.

Nutrient	System	Concentration μM	References
Inorganic			
Nitrate NO_3^-	Oceanic	0-40	Lalli & Parson (1993)
	Coastal		
Nitrite NO_2^-	Oceanic	0-3	
Ammonium NH_4^+	Oceanic	0-3	
	Coastal		
Phosphate PO_4^{3-}	Oceanic	0-3	Bronk, (1998)
Silicate $\text{Si}(\text{OH})_4$	Oceanic	0-150	
Organic	Oceanic-surface	5.8	
DON	Oceanic deep	4.3	
	Coastal	9.9	
	Estuarine	22.5	Hansell & Carlson (2002)
	Ocean deep	~ 40	
DOC	Ocean surface	~ 80	
	Coastal	150-300	
	Estuarine/Rivers	100-800	
	Coastal/Estuarine	0.05-0.98	Karl & Bjorkman, (2002)
DOP	Continental shelf	0.03-0.39	
	Open ocean	0.01-0.28	

Similarly, diatoms grow with lower Si:N and Si:C ratios under Si limitation, higher Si:N ratios under N limitation, and higher Si:C ratios under light limitation (Martin-Jézéquel et al. 2000). Another factor that alters the elemental composition of phytoplankton is the differential light dependence of photosynthesis and nutrient uptake. While photosynthetic C fixation requires light, NO_3^- can be taken up at low rates in the

dark and uptake of both NH_4^+ and Si(OH)_4 can continue through the night at rates undiminished from those in full daylight (McCarthy et al. 1996).

Bacteria have a C:N ratio lower than phytoplanktonic cells because of their high nucleic acid content. These ratios, based on bulk measurements, show a high variability (Bratbak 1985, Vadstein & Olsen 1989, Tezuka 1990, Fagerbakke et al. 1996) and range from 2.9 to 14.2 (Fukuda et al. 1998). Goldman et al. (1987) found a molar C:N:P ratio of 45:9:1 for bacteria, whereas the common molar Redfield ratio for algae is 106:16:1.

1.3.2 Organic Matter.

1.3.2.1 What is organic matter? How is it defined?

The oceans are the greatest reservoir of reactive organic carbon on earth (Hedges 1992) and more than 97 % of organic carbon in seawater is found in the operationally dissolved pool (Benner et al. 2002). Operationally, organic matter (OM) is composed of dissolved and particulate pools that are separated by a $0.45 \mu\text{m}$ boundary, regarded as being a separation between living and dead OM. In practice, dissolved organic matter (DOM) and particulate organic matter (POM) pool are separated by filtration of water samples through $0.2 \mu\text{m}$ to $1 \mu\text{m}$ cut-off size filters. Glass fibre filters that have a $0.7 \mu\text{m}$ nominal porosity are commonly used to separate DOM from POM, but small living bacteria (~ 0.2 to $0.7 \mu\text{m}$ diameter) are potentially neglected in the POM pool as living particles because they pass through glass fibre filters.

1.3.2.2 Size distribution of DOM

Even though OM has been recognised as a continuum of size in seawater (Sharp 1973), a size class distinction can be made based on physical separation by passage through membranes and filters of different pore sizes. Gustaffsson & Gshwend (1997), however, suggested that such a broad size-based definition is inadequate to understand the role of OM in marine systems (Fig. 1.03). Therefore, a size separation based on molecular weight is preferred for identifying DOM. These classes are described on passage through an ultrafiltration membrane with a 1 nm pore size (1000 Daltons (Da) cut-off).

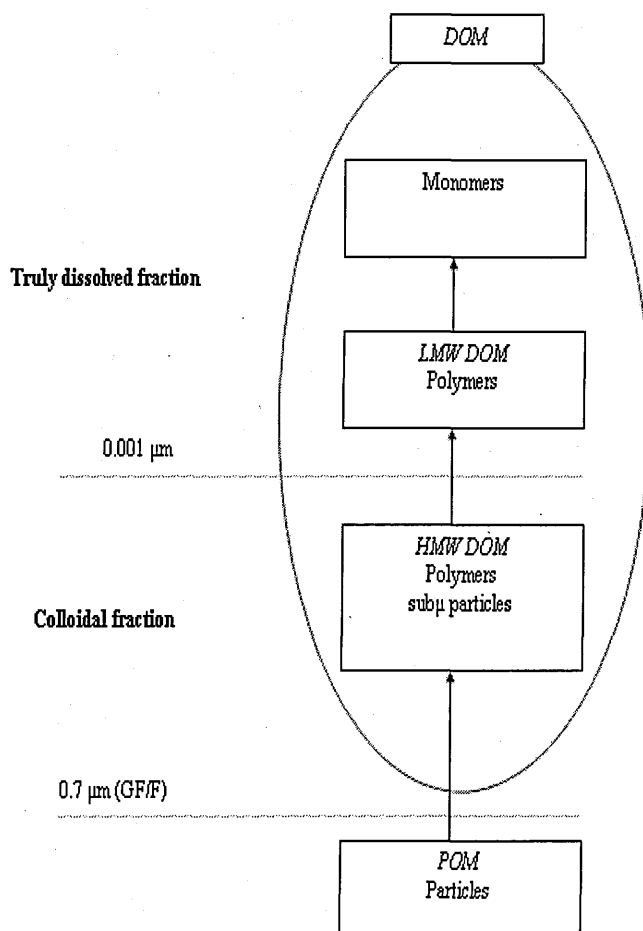


Figure 1.03. Distribution of organic matter (POM and DOM) and size fractions of DOM.

Size separation allows the distinction between High Molecular Weight DOM (HMW-DOM > 1000 Da) and Low Molecular Weight DOM (LMW-DOM < 1000 Da). Further, it is widely accepted that the majority (> 50%) of DOM is in the form of dissolved organic carbon (DOC). This DOC constitutes 60-75 % of the LMW pool in surface waters and 75-85 % of the LMW DOM pools in the deep ocean (Druffel et al. 1992, Borch & Kirchmann 1997, Skoog & Benner 1997, Benner et al. 2002). Williams & Druffel (1987) linked size separation to the lability of the DOM. Hence, because of the difference in concentration and reactivity of this HMW and LMW material; the 1kDa distinction becomes useful for understanding the cycling of DOM in seawater.

1.3.2.3 Bulk concentrations, stoichiometry and composition of DOM

Comparison between the surface and deep ocean gives a picture of the characteristics that influence DOM reactivity in oceans (Table 1.03). The major production of DOM occurs in the surface ocean, thus it is the main reservoir of fresh and reactive DOM, whilst deep waters carry old and refractory DOM (Amon et al. 2001).

Based on concentrations of DOC, DON and DOP presented by Hansell and Carlson (2002) (Table 1.03), the stoichiometry of both surface (C:N:P = 300:22:1) and deep (C:N:P = 444:25:1) waters indicates that DOM is depleted in N and P (most markedly in deep waters). This is likely to be due to remineralisation of DON and DOP in the water column. In addition, analysis of HMW-DOM stoichiometry (C:N:P of HMW DOM is very similar to C:N:P of total DOM) shows that HMW compounds are the major constituents of DOM (Benner et al. 1997, Kolowitz et al. 2001).

The molecular composition of DOM is complex and Table 1.03 gives an overview of different components found (for more details see Benner, 2002 in Hansell & Carlson, 2002 and references therein).

Table 1.03. Bulk and molecular composition of DOC (0-100 m) and deep (100-1000 m) waters (Hansell & Carlson 2002).

Chemical characteristic	Surface ocean	Deep ocean
Bulk composition		
DOC (μM)	60-90	35-45
DON (μM)	3.5-7.5	1.5-3.0
DOP (μM)	0.1-0.4	0.02-0.15
DOC/DON	9-18	9-18
DOC/DOP	180-570	300-600
Carbohydrates (μM C glucose equivalent)	10-25	5-10
Molecular composition		
Total hydrolysable neutral sugars (nM)	200-800	20-170
Total hydrolysable amino-acids (nM)	200-500	80-160
Total hydrolysable amino-sugars (nM)	42-94	4-9
Lipids (nM)	0.2-0.7	nd
Total hydrolysable neutral sugars (% DOC)	2-6	0.5-2.0
Total hydrolysable amino-acids (% DOC)	1-3	0.8-1.8
Total hydrolysable amino-sugars (% DOC)	0.4-0.6	0.04-0.07
Solvent extractable lipids (% DOC)	0.3-0.9	nd
Total (% DOC)	3.7-10.5	1.3-3.9
Total hydrolysable amino-acids (% DON)	6-12	4-9
Total hydrolysable amino-sugars (% DON)	0.8-1.7	0.2-0.4
Total (% DON)	6.8-13.7	4.2-9.4

The major constituents of LMW DOM are free and combined amino acids, neutral sugars, amino sugars and lipids. Nevertheless, it is worth noting that the molecular composition of most of the DOM pool is unknown (an average of 80%) and resides in an Unresolved Complex Mixture (UCM), identified by gas chromatography and nuclear magnetic resonance (NMR) (McCarthy et al. 1998).

1.3.2.4 Sources and sinks of DOM in marine systems

The major source of organic matter in the ocean is photosynthetic production by phytoplankton. Therefore, the amplitude of primary production controls production of DOM in the oceans. Several mechanisms are involved in DOM production including (Fig. 1.04): extra cellular release by phytoplankton (Fogg 1983, Baines & Pace 1991, Nagata 2000), sloppy feeding and excretion by grazers (Jumars et al. 1989, Caron et al. 1991, Nagata & Kirchman 1992, Nagata & Kirchman 1996, Ducklow et al. 1999, Nagata 2000), cell-lysis (viral and bacterial) (Proctor & Fuhrman 1992, Fuhrman 1999), solubilisation of particles (Smith et al. 1992b, Hoppe et al. 1993) and bacterial (enzymatic) transformation and release (Decho 1990, Tanoue et al. 1995, McCarthy et al. 1998).

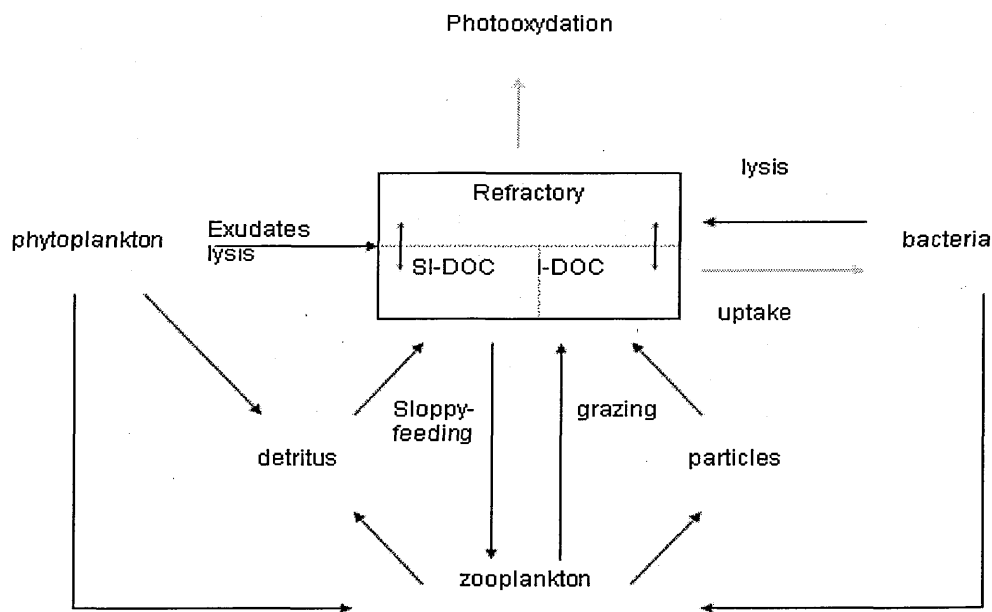


Figure 1.04. Conceptual diagram of the main production and removal processes of DOM (frame) in the marine environment. DOM pool is composed here of refractory, semi-labile (SI-) and labile (I-) DOC.

Despite a global primary production (PP) of 7 Gt C year⁻¹ (Hedges 1992), DOC concentration remains in a narrow range of 40-80 µM C, in most of the oceans. This indicates that efficient DOC removal processes occur in the sea. The main removal processes are biotic mechanisms involving heterotrophic bacterioplankton that are able to remove up to 30 % of local PP (Cole et al. 1988, Amon & Benner 1996, Ducklow et al. 1999). Bacterioplankton are able to take up LMW compounds (500-1000 Da) through their membrane (Saunders 1976), hence converting DOM to POM. Part of this organic matter is subsequently either respired to CO₂ or remineralised into its original inorganic form.

In order to assess the DOC flux going through bacterioplankton, the bacterial growth efficiency (BGE) is required. BGE can be expressed as the bacterial production (BP) divided by the sum of bacterial respiration BR and production:

$$BGE = \frac{BP}{(BR + BP)} \quad \text{equation 1.01}$$

BGE can also be approximated by the ratio of DOC converted into bacterial biomass divided by the change in extracellular DOC concentration (ΔDOC):

$$BGE = \frac{BP}{\Delta\text{DOC}} \quad \text{equation 1.02}$$

Usually, BGE is low (<20 %) for oceanic systems (Carlson et al. 1996, del Giorgio & Cole 1998) but can be very high in some coastal systems, such as the Gulf of Mexico (BGE=61 %, Kroer 1993).

The gross flux of carbon to bacterioplankton is the amount of carbon needed to support BP and is called Bacterial Carbon Demand (BCD):

$$BCD = \frac{BP}{BGE} \quad \text{equation 1.03}$$

Estimates of BP and BCD have to be used with caution because they are estimated with model compounds or precursors of nucleic acids or protein synthesis (leucine, thymidine) and require conversion factors to convert measurements into carbon units. Because of the differences in the value of conversion factors in different aquatic systems, estimation of BP, BGE or BCD leads to discrepancies between biological carbon production and consumption (Anderson & Ducklow 2001).

When DOC is not directly available, bacteria are able to break down more complex material into available compounds using exoenzymes (Decho 1990, Chróst 1992, Smith et al. 1992a, Tanoue et al. 1995, McCarthy et al. 1998). A very important diversity in enzymes probably exists in the marine environments; but only a few are considered in studies, including peptidase, alpha and beta glucosidase, and lipase. These enzymes are involved in the breakdown of simple compounds. However, studies have demonstrated the increase in bacterial growth and enzymatic activities following the peak of the phytoplanktonic blooms in mesocosms (Fajon et al. 1999) or in natural waters (Zaccone et al. 2003, Zoppini et al. 2005). In a recent study, Kirchman et al. (2004) have highlighted the relationship between enzymatic activity and bacterial taxa in coastal waters, suggesting specialisation of the different sub groups of bacteria in the degradation of organic matter.

In addition to bacterioplankton, other marine organisms can play a significant role in DOM processes. Heterotrophic flagellates, such as choanoflagellates (Marchant and Scott, 1993), and mixotrophy (Rivkin 1987, Caron 2000) have been shown as a potential sinks for marine DOM. Sherr et al. (1988) reported an active uptake of colloidal DOM by heterotrophic flagellates. Regardless, these organisms or additional

metabolic pathways, play only a modest role in heterotrophic processes on spatial and temporal scales (Carlson & Hansell 2002).

Finally, abiotic processes, such as photodegradation and/or phototransformation (Mopper et al. 1991) or absorption onto particles (Nagata & Kirchman 1996, Druffel et al. 1998) are also involved in DOM removal. Alternatively, accumulated DOM associated with particles may become entrained in interstitial water within marine aggregates (Alldredge 2000).

1.3.2.5 DOM in coastal areas

In coastal waters, an important part of the DOM comes from terrestrial sources and atmospheric deposition. Global riverine DOM inputs are estimated to be 0.25 Gt C y^{-1} (Cauwet et al. 2002). Hedges et al. (1997) showed that riverine DOM can be divided into a particulate (mostly detrital), a fine particle (colloids, N-rich) and a dissolved (N-depleted) fraction. In this dissolved pool, 70% of riverine DOM is HMW and the major constituents have a terrestrial origin (e.g. cellulose, lignin) and only a small fraction is labile enough to be degraded (Cauwet et al. 2002). In coastal areas, primary producers release C-rich DOM that accumulates in surface waters. It has been suggested that nutrient depletion does not allow heterotrophic bacterioplankton to mineralise this production (Pakulski et al. 1995, Cauwet et al. 2002), which is one of the pathways of coastal DOM export to the open ocean. Numerous studies (LeB Williams 1995, Zweifel et al. 1995, Borsheim 2000) have described a DOC accumulation in coastal areas that occurs mainly during the summer.

1.4 Sources, forms and fates of fish farming loadings.

Fish farms have been suggested as an important nutrient source in Scottish coastal waters (MacGarvin 2000). Uneaten food, fish excretion and faeces are the main fish farm inputs to the marine ecosystem. Gowen & Bradbury (1987) estimated direct food wastage of 20 % and excretions by fish (mainly NH_4^+ and urea) accounted for up to 65 % of consumed-nitrogen. In general some 85 % of phosphorus, 80-88 % of carbon and 52-95 % of nitrogen input to a marine fish culture system as feed may be lost to the environment (Hall et al. 1990, Holby & Hall 1991, Hall et al. 1992, Holby & Hall 1994, Wu 1995). These elements appear as compounds, such as proteins (46% to 51%), carbohydrates (18%), lipids (14% to 17%), vitamins, therapeutants and pigments (Gowen & Bradbury 1987).

Tett & Edwards (2002) raised three hypotheses about the effect of fish farm discharges on the marine plankton community: i) “an increase in total phytoplankton due to the stimulating affect of fish farm nutrients”, ii) “a switch in the balance of organisms due to the perturbation of nutrient ratios by these nutrients”, iii) “an increase in the toxicity of harmful algae due to the effect of these nutrients”.

Numerous studies have pointed out the potential effects of fish farm inputs for the surrounding ecosystems either *in vitro* (Takahashi & Fukazawa 1982, Arzul et al. 1996, Arzul et al. 2001) or following field studies (Jones et al. 1982, Granéli et al. 1993, Honjo 1993). As dissolved inorganic nitrogen is held to be the most important growth-limiting nutrient for phytoplankton in marine waters (Dugdale & Goering 1967) and excessive increase in dissolved nitrogen may cause hypernutrification of coastal waters, thus, dissolved nitrogen waste from fish farm might have the same effect. Dissolved inorganic compounds such as ammonium are directly released in the seawater surrounding cages via fish excreta. Inorganic phosphorus is also a feature of fish farm inputs but is likely to be more important in freshwater fish farms (Bergheim et al. 1984).

Vadstein and Olsen (1989) suggested that marine bacteria might be also phosphorus limited, hence an increase of bacterial growth if phosphorus enrichment occurs.

The major impacts of fish farm waste occurs on the seabed underneath fish cages as most of the effluent is particulate matter that sinks rapidly in the vicinity of fish pens and the impact normally stays confined within 1 km radius in poorly flushing areas (Wu 1995). The impacts on the seabed of this material are anoxic conditions, an azoic zone beneath fish cages, gas production (e.g. sulphide, methane) and ammonium release from the sediment.

Dissolved organic wastages are not well documented and have been investigated more through their effects (Wu 1995, Arzul et al. 1996) rather than a bulk parameter. Organic waste compounds have been reported to stimulate growth of particular phytoplankton species, either harmless or toxic species such as *Gyrodinium aureolum*, *Chrysochromulina polylepsis* or *Gymnodinium nagasakiense* (Wu 1995, Arzul et al. 1996, Arzul et al. 2001). Furthermore, fish excreta show variable stoichiometry and subsequently act as nutrient limiting factors for cell growth (Wu 1995, Arzul et al. 2001) and cell toxicity (John & Flynn 2002). The use of antibiotics in fish farms lead to the development of resistant bacterial population (Homer 1992). Finally, pigments and vitamins have been shown to stimulate phytoplankton species and are implicated in the toxicity of the dinoflagellate *Gymnodinium aureolum* (Gowen & Bradbury 1987).

In summary, it appears that fish farm impacts may have several effects, which depend mainly on species cultured, fish population density, feeding processes, the hydrography of exploited sites, and planktonic or benthic species, their trophic regime and sensitivity to fish farm wastes. However, very few studies have investigated the response of heterotrophic bacterioplankton or microbial community to nutrient enrichment coming from fish farms (Homer, 1992; Navarro et al. in press; Hart et al. unpublsh).

1.5 Modelling microbial food web in coastal waters

Mathematical models provide useful tools to investigate microbial food webs (Davidson 1996, Vallino 2000). Food web models are often formulated on a Nitrogen-Phytoplankton-Zooplankton-Detritus structure such as the NPZD-model of Fasham et al. (1990). Such models are useful to study the cycles of N and C in the marine environment. In addition, the recognition of the microbial loop as a major feature of marine food chains highlighted the importance of DOM cycling. “Mathematical” cycling of DOM is, however, poorly understood (Anderson & Williams 1998, Carlson & Hansell 2002), partly because bacteria are rarely included in these models (see introduction Chapter 7).

1.6 Phytoplankton in microbial food webs

1.6.1 *The common plankton groups*

The major common groups of marine phytoplankton are: the Bacillariophyceae (diatoms), the Dinophyceae (dinoflagellates), the Raphidophyceae-Cryptophyceae-Chlorophyceae (small flagellates), the Dyciophyceae (silico-flagellates), the Prymnesophyceae (coccolithophores) and Choreotrichs (ciliates). These groups cover most of the plankton found in temperate coastal waters.

1.6.2 *Plankton processes and annual succession.*

Primary producers build their own biomass (e.g. organic matter) from light, carbon dioxide and inorganic nutrients via photosynthesis, and then this organic matter becomes available to higher trophic level. Depending on the plankton species, different

nutrients are required as building blocks, e.g. diatoms specifically require silicate, in the form of orthosilicic acid (Si(OH)_4), to build their cell walls. In turn, the growth of non-diatom plankton depends more on the concentration of other nutrients such as nitrogen or phosphorus.

In temperate waters, plankton seasonality is driven by changes in light irradiance, vertical mixing of the water column, availability of nutrients and predation pressure. At the end of the winter, the water column is well mixed and contains high concentrations of inorganic nutrients. The increase of the irradiance, seawater temperature and the high availability of nutrients allow the onset of positive net photrophic production, or primary production (PP). Primary producer uptake of inorganic nutrients through permeases follows a Michaelis-Menten law that postulates that under a concentration corresponding to the constant of half-saturation of the permease, a nutrient is limiting. PP increases until one limiting nutrient is exhausted hence limiting production and/or phytoplanktonic biomass. Evolving toward summer-thermo haline stratification, the first phytoplankton bloom is replaced by other species adapted to particular nutrient levels, temperatures and light intensities.

During this time, bacteria play a key role in driving species succession by remineralising inorganic nutrients from organic matter produced and/or released during the spring event (senescent algae, exudates, particulate organic matter decomposition). These remineralised nutrients allow a second production increase that occurs in the late spring and summer. Dugdale & Goering (1967) introduced the concept of new and regenerated production according to the two types of phytoplankton growth appearing in marine ecosystems and described below. The first one, based on nutrients brought up during the winter is called “new” production and the second one supported by remineralised nutrients is the “regenerated” production.

Life strategy is important to determine the dominant species in a considered ecosystem. “r” strategy-species are able to produce numerous organisms in a short time, supporting a high population-growth and comprise competitive species in colonising new habitats. By contrast, “k” strategy species have a low reproduction rate and are found in stable environments.

High silicate concentration in seawater, poor competition for substrate, “r” life strategy, growth efficiency and/or a competitive uptake rate of silicate are cell factors which might explain why the spring bloom is usually dominated by diatoms.

1.6.3 DOM produced from phytoplankton

The quantity and quality of phytoplankton-produced DOM (see section 1.3.2.4 in this Chapter) is variable and highly dependent on the nutrient composition of the medium in which the cells are growing, the nutrient status of the cells and species-specific differences (Mykkestad 1995, Granum et al. 2002, Gilpin et al. 2004). If we are to better understand the aquatic cycling of C and N in microbial food webs it is necessary to determine the factors that govern the quality of phytoplankton DOM production and its subsequent bacterial utilisation.

Diatoms (Bacillariophyceae) are a particularly important component of the phytoplankton, contributing to 20 ~ 25 % of the global net primary production (Werner 1977). Their requirement for silicon makes them most prevalent in coastal waters (Conley & Malone 1992), where a spring bloom of diatoms is often the major feature of the annual cycle of plankton succession. When nutrients are plentiful phytoplankton are characterised by nutrient replete balanced exponential growth and typically have stoichiometric nutrient compositions in line with the Redfield ratio (Falkowski & Davis 2004). However, reduced nutrient availability, and the particular nutrient that limits

phytoplankton growth, is important in determining the cellular composition of phytoplankton in the post exponential phase. The form of the limiting nutrient is able to govern the stoichiometric balance of intracellular nutrients and the relative contribution of different organic compounds (Zingone et al. 2005). For example, phytoplankton limited by the lack of N may continue to photosynthesise and fix carbon for some time increasing the cellular carbon:nitrogen ratio from a value of approximately seven that is characteristic of balanced growth to values of 10-20 that are common for N deplete cells (Flynn et al. 1993). For diatoms the ratio of available N and Si may be of particular importance as the N:Si ratio in coastal waters (Conley & Malone 1992) and the ratio of N:Si within nutrient replete diatom cells are both often close to 1 (Brzezinski 1985). Hence relatively small changes in inorganic nutrient availability, perhaps driven by the availability of allochthonous sources of inorganic N (Aure et al. 1998, Jickells et al. 2005), may result in a change in the nutrient limiting phytoplankton growth.

For diatoms, Myklestad (1974) Myklestad et al. (1989) demonstrated that glucans (polymers of glucose) are common storage products. These glucans provide a suite of bioavailable compounds (Hama et al. 2004). However, glucan only accumulates in quantity within diatoms when growth is suboptimal. Myklestad (1974) demonstrated that this was particularly evident under conditions of N stress, reaching up to 81% of dry organic matter in one stationary phase culture of *Skeletonema costatum*. Similarly, Gilpin et al. (2004) established that the form of nutrient limitation (N or Si) governed the magnitude of glucan production by a *Skeletonema* bloom. In contrast when Si limitation of *Skeletonema* occurs, the protein synthesis pathway is not switched off because nitrogen remains available. Si limitation can potentially influence quantity (biomass) and quality (species composition) of a diatom population (Granéli et al. 1993, Davidson & Gurney 1999). However, silicification processes within diatom cells become incomplete because of the lack of silicic acid supplied to the cell. Therefore the

cell wall, or more precisely the “organic casing” containing silicon, is not sufficiently silicified and this leads to weaker cell walls and leaking cells (Martin-Jézéquel et al. 2000). DOM release can appear as a consequence of cell leakage (Maestrini & Granéli 1991) and become available to bacterial uptake.

1.6.4 The appearance of harmful species.

Of the three thousand phytoplankton recorded species, about 40 are known to have harmful properties (Sournia et al. 1991, Hallegraeff 1993). Toxic algal species sometimes dominate blooms, reaching large biomasses and leading to events called harmful algal blooms (HABs). Not all of HABs are toxic to humans but at the minimum may cause a nuisance. In Scotland, HAB events raised a general concern when they were linked to the loss of fish production in the early eighties (a red tide of *Gyrodinium aureolum*, Jones et al. 1982) and shellfish poisoning. It is recognised that changes in species succession have the potential, in theory, to allow HABs events (Tett & Edwards 2002). These authors mentioned a number of proximate causes of HAB events, including “a general and widespread change in the floristic composition of phytoplankton”, which may be related to the nutrient composition of their seawater habitat.

1.7 The current situation in Scottish coastal waters

1.7.1 Scottish Coastal Succession.

In Scottish coastal waters and sea lochs, plankton food webs follow, in general, the same picture as drawn by Fenchel (1988). Plankton succession has been described in

numerous studies from the west coast of Scotland (Wood et al. 1973, Lewis 1985, Fehling 2004). All these studies agree that Scottish coastal waters and lochs seem to experience numerous plankton blooms within the same year rather than the usual view of two annual blooms. Tett et al. (1986) recorded three blooms in Loch Striven in 1980. Fehling (2004) found six different blooms occurring throughout the year over the period 2001-2003 at a station (LY1) in the Firth of Lorne. However, Jones (1979) found only a spring and late summer autumnal bloom in Loch Creran.

Phytoplankton blooms are dominated by different species according to the particular loch or water body studied; but nevertheless some species are likely to appear recurrently in Scottish coastal waters and Scottish sea lochs. Common species found from the Scottish west coast (Table 1.04) are *Skeletonema costatum*, *Pseudo-Nitzschia seriata* group, *Thalassiosira* sp. (bacillariophyceae), *Scrippsiella* sp., *Gonyaulax* sp. (Dinophyceae), *Mesodinium rubrum* (ciliates).

1.7.2 Phytoplankton production and organic C pool in Scottish coastal water and sea lochs

Very few studies exist on the stock and dynamics of organic C in Scottish coastal waters. Jones (1979) described the seasonal phytoplankton biomass of Loch Creran as typical of coastal waters, including a nutrient limited spring bloom and a smaller summer bloom. The late summer and autumn bloom was not a regular feature of phytoplankton seasonality in Loch Creran. These observations were confirmed recently by studies of Fehling (2004). Tyler (1984), who described a C budget for Loch Creran, determined that organic C concentrations were mainly driven by phytoplankton production and river inputs in Loch Creran. This author also pointed out the highly

Table 1.04. Common plankton group and seasonality of phytoplankton in the west coast of Scotland

Time of the year	Site of study	Date of study	Most abundant species	References
March – April	Loch Striven,	1980	<i>Skeletonema costatum</i> , <i>Thalassiosira</i> sp	Tett (1986)
	Loch Etive	1970 – 1971	<i>Skeletonema costatum</i> , <i>Chaetoceros</i> sp	Wood (1973)
	Loch Creran	1982 – 1983	<i>Scrippsiella</i> , <i>Protoperidinium</i>	Lewis, (1984)
	LY1 Station (Firth of Lorn)	2001 – 2003	<i>Skeletonema costatum</i> , <i>Pseudo-nitzschia seriata</i> group, <i>Thalassiosira</i> sp.	Fehling (2004)
May – June	Loch Etive	1970 – 1971	<i>Skeletonema costatum</i> , <i>Chaetoceros</i> sp	Wood (1973)
	Loch Creran	June 1975	<i>Skeletonema costatum</i> , <i>Nitzschia seriata</i> & μ -flagellates	Jones (1979)
	Loch Striven	1980	<i>Cerataulina</i> sp, <i>Thalassiosira</i> sp, <i>Heterocapsa triquetra</i>	Tett (1986)
	Loch Creran	1982 – 1983	<i>Scrippsiella</i> sp, <i>Gonyaulax</i> sp.	Lewis (1984)
July – August	LY1 station	2001 – 2003	<i>Chaetoceros</i> sp, <i>Skeletonema costatum</i> , <i>Pseudo-nitzschia delicatissima</i> group	Fehling (2004)
	Loch Etive	1970 – 1971	<i>Skeletonema costatum</i> , μ -flagellates	Wood (1973)
	Loch Striven	1980	<i>Leptocylindrus danicus</i> , <i>Gonyaulax</i> sp	Tett (1986)
	Loch Creran	1982 – 1983	<i>Gonyaulax</i> sp, <i>Scrippsiella</i> sp	Lewis (1984)
September – October	Loch Creran	August 2002	<i>Leptocylindrus minimus</i> , <i>Mesodinium rubrum</i>	Fehling (2004)
	LY1 Station	2001–2003	<i>Mesodinium rubrum</i> , <i>Pseudo-nitzschia seriata</i> group, <i>Leptocylindrus danicus</i>	Fehling (2004)
	Loch Etive	1970 – 1971	μ -flagellates	Wood (1973)
	Loch Striven	1980	<i>Gyrodinium</i> (red tide)	Tett (1986)
	Loch Creran	1982 – 1983	<i>Gonyaulax</i> sp, <i>Protoperidinium</i> sp	Lewis (1984)
	LY1 Station	2001 – 2003	<i>Skeletonema costatum</i> , <i>Chaetoceros</i> sp, <i>Pseudo-nitzschia seriata</i> group	Fehling (2004)

dynamic character of the organic C pool in Loch Creran, calculating that the entire pool would be replaced every two to three weeks.

1.7.3 Heterotrophic bacteria in Scottish coastal waters and Scottish sea lochs

Only a hand full of studies exists for Scottish coastal waters and most of these have been undertaken in relation to fish farming (Navarro et al. in press; Leakey et al. unpublished; Hart et al. unpublished; Rogerson, 1992; Laybourn-Parry, 1992). These studies highlighted an increase in abundance and biomass of heterotrophic bacteria in the vicinity of fish cages, related to fish farm effects, through nutrients loading.

Navarro et al. (in press) also found bacterial abundance to be significantly higher at the surface (5m) than at depth (15m and 25-30m). Free living and attached heterotrophic bacterial population are mainly composed of *α-proteobacteria*, *γ-proteobacteria* and *Bacterioidetes* (M. Hart, personal communication) with an overall dominance of free living bacteria. However, it remains unclear whether or not taxonomic compositions of bacterial consortia are affected by fish farm nutrient enrichment. Leakey and Hart (personal communication) did not find differences in bacterial taxonomy between the fish farm and their control site.

1.8 Aims of this study

Marine microbial communities are clearly complex networks of interacting organisms. Phototrophic organisms build their own biomass from light, carbon dioxide and micronutrients. This potentially leads to competition for resources between phototrophic organisms, which drive the succession of different species throughout the year as resource concentrations change. In turn, higher trophic levels feed on this organic biomass that has been built by the process of photosynthesis. In addition,

photosynthetic exudates, dead cells and zooplankton excretions are used by bacteria that remineralise the organic matter, providing a source of recycled inorganic nutrients.

The dynamics of these communities (growth, succession of species, competition, grazing) are fundamental to the transfer of nutrients and energy within the whole marine environment. Nutrients available to microbial communities appear in two general forms in marine waters, organic and inorganic. Autotrophic phytoplankton uses the latter for their growth but heterotrophic organisms such as bacteria are able to assimilate both of these forms. Hence, a study of the response of microbial communities to changes in their nutrient resources is important for a better understanding of microbial food web functioning.

Many temperate coastal waters are experiencing increased anthropogenic nutrient inputs (Allen et al. 1998). Along the North and West coasts of Scotland, fish farms may be the most important source of additional nutrients in most lochs and voes. The potential exists for nutrient additions from aquaculture to modify the quality/quantity of the nutrient sources and hence the response of microbial communities within these waters. These inputs may alter the inorganic or organic nutrient stoichiometry and hence the microbial communities present.

This project seeks to address the link between organic nutrient and microbial community dynamics by:

- investigating in detail the annual changes in marine microbial communities (abundances and species) related to nutrient, on the Scottish West coast and sea lochs:

What is the importance of the different sources of organic nutrients in Scottish coastal waters and fjordic sea loch?

Is the dynamics of microbial communities different between open coastal water station and restricted exchange fjordic system?

How do microbial communities respond to these different sources of nutrients?

- conducting perturbation microcosm experiments to study the influence of changes in inorganic and organic nutrients on microbial community productivity and composition:

Does the stoichiometry of organic nutrients influence the metabolism of bacterioplankton and the subsequent transfer of these nutrients to higher trophic level?

Can inorganic nutrient have indirect effects on the DOM pool and the bacterioplankton?

What is the limiting factor of heterotrophic productivity in Scottish coastal water?

Do bacteria really compete with phytoplankton for inorganic resources?

- using the data collected, to parameterise and test existing mathematical models to simulate the influence of changes in organic nutrient concentrations on microbial community dynamics.

- CHAPTER 2 -

Materials and methods

2.1 Sampling

Sampling was carried out on board of the R/V Seol Mara (SAMS research vessel) on the west coast of Scotland (Fig. 2.01) at three stations (Table 2.01), LY1, FF (Fish farm) and C5 (Barcaldine). Each station was sampled at three depths (3, 10, 15m) from 12/03/2004 to 21/2/2005; then only two depths (3, 10 m) were sampled from 7/3/2005 to 16/9/2005. There were 32 sampling occasions in total.

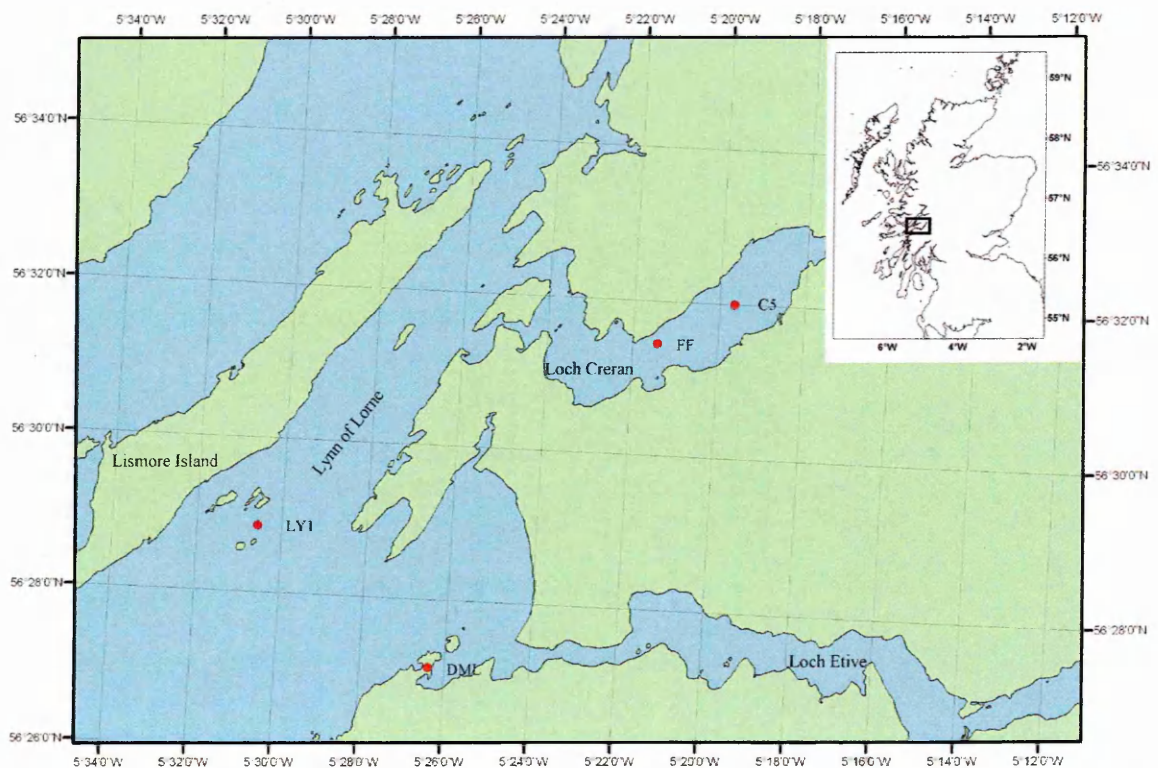


Figure 2.01. Map of the location of Loch Creran (stations FF and C5) and the Firth of Lorne (station LY1).

From each depth, two times one litre water sample was collected using one litre NIO bottles, launched from RV Seol Mara. Seawater was then transferred into cleaned thermos flasks. These allowed transport of the samples in the dark, at ambient temperature, to the laboratory, where samples were processed for chemical and biological analyses within 3 to 4 hours after collection.

Table 2.01. Names, position, average depth of stations sampled.

Station	Position	Average depth (m)	Depth sampled (m)
LY1	56° 28.9 N, 05° 30.1 W	52	3,10,15
Loch Creran:			
Fish farm	56° 31.5 N, 05° 20.9 W	22	3, 10, 15
Barcaldine (Station C5)	56° 32.1 N, 05° 19.4 W	25	3, 10, 15

2.2 Physical variables.

Conductivity, temperature and depth (CTD) profiles of the upper 20 m of the water column were recorded with a Seabird CTD probe (model Seabird 19, Sea-Bird Electronics, USA). Contour plots of time series were constructed using the Sigmaplot 9 software (Systat software Inc.).

Secchi disk depths were recorded at each site using a Secchi disk and converted into irradiance according to Holmes (1970). The coefficient of light extinction, K_D , was estimated with the formula:

$$K_D = \frac{1.44}{z_{SD}}$$

equation 2.01

where 1.44 was the coefficient attributed to turbid coastal water and Z_{SD} the Secchi disk depth measured. 10 % irradiance depth was derived from the second formula:

$$Z_x = \ln \times \left(\frac{100}{X} \right) \times \frac{1}{K_D} \quad \text{equation 2.02}$$

where X was the percentage of the light attenuated at the depth Z , (fixed at 10 % here), Z , the depth of 10% irradiance and K_D were replaced from the first equation. Tyler (1968) showed that the sum of K_D and α (beam transmittance) could be estimated with a Secchi disk.

2.3. Chemical variables.

2.3.1 Inorganic nutrient concentrations.

Inorganic nutrients were analysed by colorimetric analysis according to Grasshof (1970) using an autoanalyser (Lachat Quick Chem 8000, Hach Lange, Colorado, USA) and using a standard flow injection method. Sub-samples (30 to 60 ml) for inorganic nutrients (ammonium, nitrate, nitrite, phosphate and silicate) were collected from filtrate (A/E glass fibre filters, Pall Gelman) and stored frozen at -20°C in polycarbonate bottles. Samples were defrosted overnight prior to analysis and transferred into cleaned plastic (polycarbonate) tubes designed for the analyser.

Standard stock solutions of nutrients were prepared in deionised water on the day of each batch analysis. A mixed working solution of ammonium, nitrate, phosphate and silicate standards was used. Nitrite standards were made separately because they would otherwise interfere with nitrate analyses. Each calibration (mix and nitrite) was conducted with five dilutions at 20, 34, 50, 100 and 250 times and a blank run with

deionised water (ELGA system). The chosen range of concentrations of standard depended on the type of samples analysed (experiments with additions of inorganic nutrient or natural samples). Calibration factors were obtained using first or second order polynomial regression (Omnion software, Lachat Instruments, USA). Prior to calibration and batch analysis, a dye test (green dye) was performed to set up flow and valves timing.

Duplicated aliquots (8 ml) from sub-samples (filtrates) were used for analysis. A drift correction was applied using times 50 dilution standards run in triplicate at the beginning and the end of each batch. Incremental drift correction, using the average difference of the drifts, was then applied to samples. A salt correction was also applied to each batch following sample analysis and subtracted from each value. Salt correction was performed by removing critical reagents for ammonium, nitrate, phosphate and silicate reactions (nitroprusside, sulphanilamide alone, molybdate and stannous chloride respectively). These reagents were replaced with deionised water, which prevented coloured-complex formation. Salt correction for nitrite analysis followed exactly the same protocol.

2.3.2 Organic nutrient concentrations.

2.3.2.1 Dissolved organic nutrient concentrations.

Dissolved organic nutrient were analysed by high temperature catalytic oxidation (HTCO) (Sugimura & Suzuki 1988) reviewed by Cauwet (1994). Calibrations were performed at the beginning of the day with a mix of glycine ($C_2H_5NO_2$, VWR, stock concentration 80 mM C, 12.25 mM N) and potassium hydrogen phthalate ($C_8H_5PO_4$, Nacalai Tesc, stock concentration 20 mM) giving a stock solution of 100 mM C and 12.25 mM N. Four-point calibrations including a blank of deionised water

(ELGA system), were performed on the Shimadzu TOC 5000A. Three dilutions from stock solution were calculated in order to cover the likely range of concentrations of the samples analysed. Dilutions were made in 100ml volumetric flasks with deionised water and using a very clean glass syringe to measure stock solution volumes. Calibrations give an average of r^2 of 0.99 for both C and N ($n = 24$).

Duplicated sub-samples (10 ml) were filtered through precombusted (24 h, 450 °C) glass fibre filters (Whatman GF/F 25 mm, 0.7 μ m effective cut-off), with a glass syringe and filter cartridge, and using polyethylene gloves. Each filtered sub-samples was collected in a precombusted glass ampoules, preserved with orthophosphoric acid (35 μ l Analar 85 % H_3PO_4 per 10 ml sample), sealed with a flame and refrigerated (4-6 °C) in the dark, until analysis. DOC was analysed on a Total Organic Carbon analyzer (Shimadzu TOC-5000A) with platinum catalyst (0.5% on alumina), carrier gas as O_2 (N5 grade, 150 ml/min), coupled to an NDIR (Non-Dispersive infra-Red) detector. Total Dissolved Nitrogen (TDN) was analysed with a nitric oxide Analyser (Sievers 280i NOA) using high temperature oxidation to nitric oxide (NO) and detection by chemiluminescence (Walsh 1989, Hansell et al. 1993, Sharp et al. 2004). Dissolved organic nitrogen (DON) was obtained by subtraction of total inorganic nitrogen (see section 2.3.1 above) from TDN.

2.3.2.2 Liquid chromatography analysis of DOM

The carbohydrate from the DOM produced by *Skeletonema costatum* (see Chapter 5) were extracted from cell free spent medium of algal culture. This was performed by adding two volumes of cold ethanol to the medium collected, followed by subsequent recovery, extensive dialysis with ELGA water and freeze drying of the precipitated material. To characterise the carbohydrate molecular weight profiles in

precipitated material. To characterise the carbohydrate molecular weight profiles in these extracts, high-performance liquid chromatography (HPLC) was performed with an Agilent 1100 chromatograph (Agilent Technologies) equipped with a refractometer and diode-array UV detector. A PL gel filtration chromatography column (Polymer Laboratories; 7.5 x 300 mm) was used at 30°C to identify the high molecular weight (>50 kDa) content in the extracts. The mobile phase used was 0.1M NaNO₃ and the flow rate 0.6 ml min⁻¹. Dextran standards of *M_r* range 50–1,400 kDa (Sigma) were used to calibrate the column for molecular weight estimation. Additionally, in order to determine for the presence of free mono- and di-saccharides in the *S. costatum* extracts, samples were run through a Polyamine II column (YMC-Pack; 4.6 x 250 mm) at 30°C, with acetonitrile:water (70:30) as the mobile phase and flow rate at 0.8 ml min⁻¹.

2.3.2.2 Particulate organic carbon and nitrogen concentrations.

Seawater samples (100 ml) were filtered onto a glass fibre filter (Whatman GF/F, diameter: 13 mm, precombusted for 24 hours at 450°C) and stored frozen at -20°C. Prior to analysis, filters were oven dried at 60 °C overnight and folded into tin disks. Samples were analysed with an ANCA NT prep system coupled with a 20-20 Stable Isotope Analyser (PDZ Europa Scientific Instruments, Northwich, UK). Calibration was performed using a solution of isoleucine (L- Isoleucine, Europa STD) at concentrations of 1 µg N and 6 µg C. Standards were placed in tin caps (with Chromosorb W, PDZ Europa ltd) and oven dried at 60°C overnight. Calibrations were performed with series of standards from 5 to 200 µg N (25– 1029 µg C) run at the beginning of each batch. Calibration curves give a *r*² of 0.99 for both C and N (n=15).

2.3.3 Chlorophyll *a* concentrations

Chlorophyll *a* (chl *a*) concentrations was measured by fluorometry (Jeffrey & Humphrey 1975). Immediately after returning to the laboratory, 500 ml sub-samples were filtered in duplicate on a 25 mm glass fibre filter (type A/E, Pall Corporation, Portsmouth, UK) and stored frozen at -20°C in ependorff tubes. Prior to analysis filters were thawed in 15 ml centrifuge tubes and pigments were extracted over night in the dark at 4°C with 8 ml of 90% acetone (VWR). Filters were sonicated for 1 minute and after subsequent centrifugation (3000 rpm for 5 min) chl *a*. was measured with a Turner TD-700 fluorometer (Turner Design, Sunnyvale, CA, USA). The fluorometer was calibrated using chl *a* concentrations extract from spinach (Sigma, stock concentration 3.19 mg L^{-1}). Prior to this, chl *a* concentration was first verified using a scanning spectrophotometer (Nicolet evolution 300, Thermo Electron Corporation, Cambridge, UK). Absorbance at 663-750 nm was measured for unacidified and acidified (50 μL HCL, BDH, AnalaR) and concentration was calculated as follows:

$$[\text{Chl}a.] = A \times K \times (\text{Abs}_0 - \text{Abs}_a) \quad \text{equation 2.03}$$

where *A* is the inverse coefficient in 90 % acetone (using the value of Jeffrey and Humphrey, 1975) and *K* is the standardised acid factor (*R*) of the chl *a* extinction coefficient (87.7 g.cm^{-1}) over pheophytin extinction coefficient (51.2 g.cm^{-1}). The Turner fluorometer was then calibrated using a 0.1 and 0.2 dilution of stock chl *a* standard. Three readings of unacidified and acidified subsamples of both dilutions were made. Blanks were measured with 90% acetone only and HIGH and LOW solid standard (SST) measurements were taken to account for drift. SST was a fixed standard from which readings (high “H” and Low “L”) are taken during calibration. It was,

therefore, used as reference to correct drifts related to the fluorometer. A series of ten samples were measured including a 90% acetone blank and High SST at the beginning and end of these ten samples. Chl *a* concentrations in samples were calculated according to:

$$[Chla.] = K \times \left(\frac{F_m}{(F_m - 1)} \right) \times (F_b - F_a) \times \left(\frac{V_{extracted}}{V_{filtered}} \right) \quad \text{equation 2.04}$$

where K is the calibration constant, F_b is the fluorescence before acidification, F_a is the fluorescence after acidification, F_m is the max acid ratio (F_b/F_a) of pure chl *a*, $V_{extracted}$ is the extraction volume and $V_{filtered}$ is the volume filtered.

Similarly to chl *a*, pheopigment concentrations were calculated according to:

$$[Pheo.] = K \times \left(\frac{F_m}{(F_m - 1)} \right) \times (F_m \times F_b - F_a) \times \left(\frac{V_{extracted}}{V_{filtered}} \right) \quad \text{equation 2.05}$$

An average value for the 90% acetone blank (before and after 10 samples run) was subtracted from each value and an incremental drift correction was applied to each series of 10 samples using HIGH SST values. chl *a* concentrations were averaged from duplicate values for each sample.

2.3.4 Primary production and extracellular organic carbon (EOC)

The following method is specific to Chapter 6 and describes the method as used for the experiment. Primary production (PP) was measured using the ^{14}C radioisotope method. Measurements of remaining radioactivity within the filtrate from the PP

measurements allowed estimation of extracellular releases of dissolved organic C (EOC) by phytoplankton cells (Larsson & Hagstrom 1979).

For each experimental replicate, seven 50 ml sub-samples were transferred in 60 ml polycarbonate bottles as follows: four sub-samples for incubations in the light (two duplicates stopped at t_{24} and two duplicates stopped at t_{48}), two sub-samples for incubations in the dark (one stopped at t_{24} , one stopped at t_{48} , wrapped with tin foil) and one sub-sample as a blank; of which two replicates and one dark bottle were stopped by filtration at 24 and 48 hours. 2 μCi of ^{14}C labelled bicarbonate ($\text{NaH}[^{14}\text{C}]\text{O}_3$) were added to all bottles except blanks. Aliquots of 200 μl were taken from 12 random bottles for initial radioactivity check and these were placed in scintillation vials with 10 ml of scintillation cocktail (supermix:carbosorb:mQ water, v:v:v). Simultaneously, blanks were filtered through a 0.2 μm polycarbonate filter and were later processed as incubated samples (see below). All the bottles were incubated under exactly the same conditions of temperature and light (see section 6.2.2, this Chapter).

After incubation (24 and 48 hours), all sub-samples were filtered through a 0.22 μm polycarbonate membrane (Whatmann, 47 mm) and acidified in a dessicator for at least 12 hours with 12 N HCl fumes to remove inorganic radiolabelled C. Filters were then placed in scintillation vials with 4.5 ml of scintillation liquid (Optiphase II). Filtrates were collected and an aliquot of 5 ml was then placed in a scintillation vial with 1 ml of 6 M HCl; this was then agitated on a planetary shaker for at least 48 hours. 5 ml of filtrate were transferred into scintillation vials and they received 15 ml of Supermix scintillation liquid.

Radioactivity was measured using a Wallac LKB Rackbeta 1219 Spectral liquid scintillation counter. Total CO_2 concentrations were estimated from salinity measurements (Sorokin 1987). Net C uptake rates were corrected for dark and initial

fixation (blank). There were no PP and EOC values available at t_{zero} because these are determined over a period of 24 and 48 hours.

2.4 Biological variables.

2.4.1 Planktonic community composition.

2.4.1.1 Phytoplankton composition and abundance:

Approximately 200 ml seawater sub-samples were preserved with Lugols Iodine (1% final concentration) and stored in the dark in 250 ml amber glass bottles. Phytoplankton cells from all samples were counted by inverted microscopy using the Utermöhl method (Utermöhl 1931). 10 or 50 ml of lugol-fixed aliquots were settled in 10 or 50 ml Utermöhl chambers respectively for 24 hours prior to the counts. The area of at least half a chamber, or 100 cells of the most abundant species, were counted at 200-400 magnification using an Axiovert S100 inverted microscope (Zeiss, Jena, Germany). Counting the whole chamber fixed the limit of detection at 20 individuals L^{-1} . Phytoplankton analyses were carried out by Ms E. Mitchell.

2.4.1.2 Pico- and nano-plankton abundance:

Pico- and nano-plankton were determined by epifluorescence microscopy according to Porter & Feig (1980) and Sherr et al. (1993). Seawater sub-samples (100 ml) were fixed with glutaraldehyde (final concentration 1%) and stored in brown plastic bottles in a cold room (4-6 °C). Enumeration of phototrophic and heterotrophic nanoplankton (PNAN & HNAN), cyanobacteria and bacterioplankton (heterotrophic bacteria and archaea) was performed using a Zeiss Axioskop2 mounted with 02 (UV excitation), 09 (blue excitation) and 20 (green excitation) filter blocks.

HNAN and PNAN were counted using 15 ml of glutaraldehyde fixed aliquots stained with DAPI (4'-6-Diamidino-2-phenylindole, $5\mu\text{g}.\text{ml}^{-1}$) for a period of 5 to 10 minutes and filtered onto a white polycarbonate filter (Whatman, 25 mm, 0.8 μm porosity). The filtration funnel was rinsed twice with 0.2 μm filtered deionised water (ELGA system). Filters were stored on slides and frozen at -20°C until counting. Enumeration was performed by epifluorescence microscopy (Sherr et al. 1993) using a Zeiss Axioskop2 at x1000 magnification and 25 to 50 fields of view (FOV) were counted. Nanoplankton was identified by their blue fluorescence under UV light, and HNAN were distinguished from PNAN by the absence of chlorophyll-a autofluorescence and red fluorescence of chlorophyll under green illumination.

2.4.1.3 Cyanobacteria

Cyanobacteria abundance was determined according to Porter & Feig (1980). Aliquots (5 ml) of glutaraldehyde fixed seawater (1% final concentration) were filtered onto a white polycarbonate filter (Whatman, 25 mm, 0.2 μm porosity) and stored on slides and frozen at -20°C . Cyanobacteria were enumerated by epifluorescence microscopy (Zeiss, Axioskop2) and identified by their orange fluorescence under blue light illumination. 30 FOV were enumerated for each filter.

2.4.1.4 Bacterioplankton abundance

Bacterioplankton was determined according to Porter & Feig (1980). Aliquots (5ml) were stained with DAPI for 5 to 10 minutes and filtered onto black polycarbonate filters (Whatman, 25 mm, 0.2 μm porosity). Filters were stored on slides, frozen at -20°C

C and observed at x1000 magnification under fluorescence illumination using a Zeiss Axioskop2. 30 FOV were enumerated for each filter.

2.4.2 Bacterioplankton taxonomic composition

Bacterial community structure was analyzed by fluorescence in situ hybridization (FISH). Bacteria from fixed samples (molecular grade paraformaldehyde, 4% final concentration) were collected onto 0.2 μm white polycarbonate membranes (25 mm diameter, Nucleopore) by filtering 5 ml under gentle vacuum and washing with 0.2 μm filtered ELGA water. Until further processing, the filters were stored in parafilm-sealed Petri dishes at -20°C . FISH analysis was performed according to the protocol of Pernthaler (2001). Each filter was cut into six sections and hybridized for 90 minutes at 46°C in individual (for each probe) equilibrated chambers. The oligonucleotide probes specific of Eubacterial groups used are summarized in Table 5.03. All probes were commercially synthesized (TAG, Newcastle, UK) and labelled with CY3. Unlabelled competitors probes were used with GAM42a and BET42a to prevent mis-annealing of non-target organism.

The probe EUB338 was used to maximally target the Eubacteria, and nonsense probe NON338 was used to assess background fluorescence counts. After hybridization the filter sections were rinsed with a washing buffer at 48°C for 15 minutes and rinsed in ice-cold deionised ELGA water. Filter sections were mounted on microscope slides in Vectashield-DAPI medium and stored at -20°C until enumeration.

Table 2.02. Oligonucleotides probes sequences used to detect the bacterial group in this study.

Probe	Specificity	Sequence (5'-3') of probe	Target site ^a (rRNA position)	% Formamide	References
EUB338	Bacteria	GCTGCCTCCCGTAGGAGT	16S (338-355)	35	(Amann et al. 1990)
NON338		ACTCTACGGGAGGCAGC	16S (338-355)	35	(Wallner et al. 1993)
ALF968	Alpha subclass of Proteobacteria	GGTAAGGTTCTGCGCGTT	16S (968-986)	35	(Neef 1997)
BET42a	Beta subclass of Proteobacteria	GCCTTCCCACTTCGTTT	23S (1027-1043)	35	(Manz et al. 1992)
GAM42a	Gamma subclass of Proteobacteria	GCCTTCCCAACATCGTTT	23S (1027-1043)	35	(Manz et al. 1992)
CF319a	Cytophaga-Flavobacterium cluster of CFB phylum	TGGTCCGTGTCTCAGTAC	16S (319-336)	35	(Manz et al. 1996)

^a E. coli numbering

The relative abundance of hybridized cells was estimated as a ratio of hybridized cell counts to counts of DAPI-stained cells using epifluorescence microscopy. Cell enumeration was performed at x1000 magnification with an Axioskop2 epifluorescence microscope (Zeiss) under fluorescence illumination and equipped with a 20 filter block (green excitation). Hybridised bacterial cells were identified by their orange fluorescence under green light and enumerated from 20 FOV for each filter section.

2.4.3 Bacterial production

Bacterial production was determined from [*methyl*-³H]thymidine incorporation (Fuhrman & Azam 1982). Working solutions (100 nM thymidine) were prepared periodically from [*methyl*-³H]thymidine stock solution (Amersham Biosciences TRK 418, specific activity 50 Ci.mmol⁻¹, isotopic concentration 0.5 µCi.ml⁻¹). Clean sterile Falcon tubes were weighted empty and after addition of 0.5 ml of radiolabelled thymidine. The stock solution of radiolabelled thymidine was sampled with a sterile syringe through a plastic cap. This procedure prevented the microbial contamination of the whole stock solution. The sampled volume was weight corrected and sterile deionised water (ELGA system) was added to achieve an accurate concentration of the working solution (100 nM thymidine). All working solutions used for this work were checked by mixing triplicate 100 µl aliquots of working solution with 10 ml scintillation cocktail for counting by scintillation counter.

Three aliquots (9.9 ml) of seawater were incubated in triplicate with 100 µL of a working solution of [*methyl*-³H]thymidine (Amersham Biosciences TRK 418, specific activity 50 Ci.mmol⁻¹, isotopic concentration 0.5 µCi.ml⁻¹). One additional aliquot was killed with 10ml of ice-cold 10% trichloroacetic acid (TCA) and was used as T_{zero}. After one hour of incubation, the remaining three sub-samples were stopped with ice cold

10% TCA and filtered onto white polycarbonate filters (Whatman, 25 mm, 0.2 μm porosity) mounted onto a glass fibre filter (type GF/A, Whatman, 25 mm, 1.1 μm nominal cut-off). Both polycarbonate and glass fibre filters were soaked in a petri dish containing a solution of non-radioactive thymidine (SIGMA, 1 mM concentration) before filtration. Filtration funnels were rinsed three times with 5 ml of ELGA water and 5 ml of 95% ethanol for each sample. Filters were dried over night in 10 ml plastic scintillation vials, prior to the addition of 10 ml of scintillation liquid (Ultima Gold, Packard). Scintillation was counted using a LKB Wallac liquid scintillation counter (LKB Wallac, 1219 RACKBETA). Efficiency of counting was determined by the external standard channel ratio method. [*methyl*- ^3H]thymidine incorporation was calculated from counts (corrected for quenching) using isotope specific activity corrected for decay (Bell 1993). Carbon equivalents were calculated using the value of 30.2 fgC cell $^{-1}$ (Fukuda et al. 1998).

2.4.4 Thymidine conversion factor: dilution experiment

Dilution experiments were used to estimate empirical thymidine conversion factors to convert isotope incorporation into cell growth (Kirchman & Ducklow 1993). These experiments were carried out at three different times of the year; spring, summer and winter. Seawater samples were collected with 1 L Niskin bottles at LY1 and the fish farm site during routine sampling and stored in thermos flasks from 10 m depth. One part of seawater was diluted (1:9 dilution) with nine parts of 0.2 μm filtered seawater (Vacuap, PAL company, 47 mm, 0.2 μm ; under gentle vacuum). Triplicated sub-samples were incubated over 24 hours in the dark at ambient temperature, and bacterial abundance and production was assayed every 6 hours. Aliquots from each triplicate (5 ml) were fixed with glutaraldehyde (1% final concentration) for bacterial abundance by

DAPI staining (see section 2.4.1.4, this Chapter) and, simultaneously, aliquots from each triplicate (9.9 ml) were used to determine radio-labelled thymidine incorporation method over one hour (see section 2.4.2, this Chapter).

2.5 Statistical analysis

Data sets, from field studies or experimental work, were analysed statistically in order to verify trends, observations and patterns. Most of the analyses used were multivariate. Multidimensional analysis (MDS) was used to illustrate population assemblages in different samples, Principal Component Analysis (PCA) was utilised to highlight similarities between stations and environmental factors, and Redundancy Analysis (RDA) was performed in order to determine relationships between biological parameters and physical and chemical factors measured at each station.

Student's t-test, normality test, ANOVA and PCA were performed with the free software R (Genuine Licence) using appropriate statistical packages. MDS was performed with the software PRIMERTM (Plymouth Routine in Multivariate Ecological Research, Plymouth, UK). RDA was carried out using the CANOCO software (Leps & Smilauer 2003).

2.5.1 Error propagations:

Propagation of standard errors (σ) or standard deviation (σ^2) were calculated according to:

- If additions or subtractions: $\sigma_{A+B} = \sqrt{\sigma_A^2 + \sigma_B^2}$ equation 2.06

- If multiplications or divisions: $\sigma_{A \times B} = \overline{A \times B} \times \sqrt{\frac{\sigma_A^2}{A^2} + \frac{\sigma_B^2}{B^2}}$ equation 2.07

2.5.2 Normal distribution

In order to test the distribution of variables, a Shapiro-Wilk test was used (Shapiro & Wilk 1965). In statistics, the Shapiro-Wilk test tests the null hypothesis that a sample x_1, \dots, x_n came from a normally distributed population. Alternatively, if a normal distribution of the variable was rejected by the Shapiro-Wilk test, a non parametric test was applied, such as Mann-Whitney U.

2.5.3 Student test

A student t-test tests the null hypothesis that the means of two normally distributed populations are equal. Given two data sets, each characterized by its mean, standard deviation and number of data points; it is possible to use the t-test to determine whether the means are distinct, provided that the underlying distributions can be assumed to be normal. The assumptions of such a test are that the data are normally distributed, homosedastic, the equal of variance, and that the samples may be dependent or independent. Variables were tested for null hypothesis of no difference between sites (LY1, FF or C5) or depth (3 and 10 m) using a Student's t-test.

2.5.4 Multidimensional scaling analysis (MDS)

MDS is used to construct a map of configuration of samples, in a specified number of dimensions, which attempts to satisfy all the conditions imposed by a rank (dis)similarity matrix (Clarke & Warwick 2001). On the ordination the placement of samples reflects the similarity of their biological communities. The distances between samples on the ordination attempts to match the corresponding dissimilarities in

community structure. Hence, close points have similar communities, while samples that are far apart have few species in common or different abundance of the same species (Clarke & Warwick 2001).

2.5.5 Principal Component Analysis (PCA)

The method of principal component analysis (PCA) was first introduced by Pearson (Pearson 1901). PCA is a technique to approximate high-dimensional information in a low-dimensional plot. Data from a dissimilarity matrix of the environmental data sampled at different stations are used to define the positions of samples in relation to axes representing the full set of measured environmental parameters (Clarke & Warwick 2001). The distance between two stations in the plot is a measure of their dissimilarity and the further two points are apart, the more the environmental parameters at each station differ from each other.

2.5.6 Redundancy Analysis (RDA)

Redundancy analysis (Rao 1964) can be described as a series of multiple linear regressions, using linear model of relationships among environmental parameters and between biological and environmental variables. RDA may also be considered as a constrained form of PCA, which identifies trends in the scatter of data points that are maximally and linearly related to a set of constraining (explanatory) variables (Makarek & Legendre 2002). RDA is used to investigate the strength of relationship between measured environmental factors and individual species within a multivariate data set. Similarly to PCA, RDA uses Euclidean distances. A Monte Carlo test is performed to test the significance between environmental variables and the biotic

composition. RDA was carried out at LY1 and FF for phytoplankton data in order to investigate environmental variables affecting significantly the abundance of species present at both stations.

- CHAPTER 3 -

Organic nutrient and microbial community dynamics

- Field Study -

3.1 Rationale

It is now recognised that tight coupling between phytoplankton and bacteria can exist in coastal waters, as well as in the open ocean (Fuhrman et al. 1980). In coastal areas, extra sources of available organic nutrients may alter this relationship. Further, phytoplankton and bacteria may compete for N resources, as both are able to take up inorganic (Dugdale & Goering 1967, Wheeler & Kirchman 1986, Kirchman 2000) and organic nitrogen (Fuhrman & Azam 1982, Antia et al. 1991).

Dissolved organic carbon and nitrogen (DOC and DON) may be of major importance to microbial dynamics in coastal waters but their concentrations, sources and sinks remain poorly quantified in comparison to inorganic nutrients. DON can be a significant fraction of total dissolved nitrogen (TDN) and may represent 13% to 18% of nitrogen in coastal and estuarine waters (Berman & Bronk 2003) of which 12 to 72% is considered to be bioavailable (Allen et al. 2002). Similarly, DOC may provide a labile C source to coastal microbial communities and hence, both forms of organic nutrients (C and N) are important for driving bacterial abundance (BA) and production (BP) (Ducklow 2000).

In comparison to phytoplankton abundance and production, time series estimates of bacterial abundance and particularly production are relatively rare in coastal waters (see review by Ducklow 2000). Uptake of radio-labelled thymidine is the common

method of choice for obtaining the productivity estimates that are particularly important for inter-comparison between ecosystems. However, productivity determinations are potentially compromised by the use of literature rather than *in situ* conversion factors (Ducklow 2000). An assessment of the calculations used to determine bacterial productivity is therefore required.

In addition to autochthonous (e.g planktonic) production of DOM, the coastal marine environment receives various extra inputs of organic nutrients from sources such as rivers or through anthropogenic activities. Despite its recognised refractory character, DOM from riverine inputs is partly degraded in coastal waters with turn over time of days to years (Cauwet et al. 2002); this indicates its (at least partial) availability to bacteria. Additionally, in Scottish coastal waters aquaculture, in particular fish farming, often provides a point source of both inorganic and organic nutrient (Arzul et al. 1996) which may perturb microbial community dynamics.

Finally, the manner in which bacterial communities respond to the quality and quantity of dissolved organic nutrients may influence trophic transfer within microbial communities. Heterotrophic nanoflagellates (HNAN) and ciliates are the main grazers of bacteria in marine environments (Sherr et al. 1986, Sherr & Sherr 1991, Strom 2000) and hence remove a significant proportion of bacterial production. Grazers of bacteria make this organic matter available to higher trophic levels (e.g larger grazers), which might not otherwise have access to this source of energy (Strom 2000). However, it remains unclear whether or not this grazing link is efficient in the transfer of energy through microbial food webs (Thingstad et al. 1997) and how this transfer may vary between locations.

3.2 Objectives

The aims of this part of the study were:

- 1) to assess the concentrations of dissolved organic nutrients (DOC and DON) throughout a full annual cycle at three contrasting locations in Scottish coastal waters,
- 2) to concurrently determine bacterial abundance (BA) and production (BP) at these sites and how these relate to dissolved organic matter (DOM),
- 3) to assess the influence of different, direct and literature based, methods of calculating bacterial production,
- 4) to determine the role of grazers in controlling bacterial population and trophic transfer of energy in microbial food webs.

To achieve these goals, a comprehensive set of biological state (chl α , bacterial abundance, heterotrophic and phototrophic nanoflagellates abundance, dinoflagellates and diatoms abundance) and rate (bacterial production) variables were determined over a two year period at each site, along with physical (temperature, salinity, density, currents) and chemical (inorganic and organic nutrients, particulate organic matter) variables.

3.3 Description of the sampling site

Three contrasting locations were sampled to allow the comparison of annual trends in microbial community composition in relation to DOM concentrations as well as other nutrients and water column properties (see Fig. 2.1, in chapter 2). Sites were chosen in order to investigate an *a priori* hypothesis of the stimulation of bacterial activity by the point source nutrient input from a fish farm within a sea loch. One site was therefore located near to a fish farm so that comparison could be made with a non

anthropogenically impacted site in the same loch (but which would be expected to receive significant riverine nutrient loading) and a further open water site outside the loch.

Sampling was conducted within Loch Creran and the Firth of Lorne (see Chapter 2 for details). Loch Creran was chosen because it is, in its dimensions, freshwater input and tides, close to the “unrealised” typical Scottish sea-loch (Landless & Edwards 1976). It experiences anthropogenic inorganic and organic nutrient input from a fish farm located approximately in the middle of the main basin of the loch. Finally, Loch Creran is also readily accessible by boat from the SAMS laboratory.

Two sites were sampled within Loch Creran, a station close to a fish farm (FF) and station C5, described in previous studies (eg. Tett & Wallis 1978, Jones 1979, Grantham 1983a) and used in this study as a non-anthropogenically impacted site within the loch. Most of the historical data discussed below comes from this C5 station.

Open water station LY1 in the Firth of Lorne was chosen to provide a contrasting station to those with Loch Creran, *a priori* less influenced by organic inputs and because previous intensive studies (eg. Tett & Wallis 1978, Jones 1979, Grantham 1983a, Fehling 2004) have provided considerable background data regarding inorganic nutrients and phytoplankton abundance at this station.

3.3.1 LY1 and Loch Creran: historical comparison.

3.3.1.1 LY1

Station LY1 is located near the Greag Isles, Firth of Lorne, lat. 56°28'.9 N, long. 5°30'.1 W, with a depth of 52m. LY1 station has previously been studied and used as a control site for studies in Loch Eil and Loch Creran. It is directly influenced by the outflow from Loch Etive and Loch Creran. However analysis of CTD and

phytoplankton data from a transect from Loch Spelve to Loch Creran suggested that it is typical of open coastal waters (Fehling et al. 2006). Monthly inorganic and organic nutrient concentrations, salinity, and temperature data from 1979 to 1981 (Grantham 1983a, b), and from 2001 to 2003 (Fehling 2004) exist for this station. Organic nutrients were also analysed in the Firth of Lorne by Solórzano & Ehrlich (1979). Tett et al. (1981) provided, from this station, one of the few studies of the annual cycle of phytoplankton in Scottish coastal waters. This was complemented by a study carried out by Fehling (2004) that generated data on inorganic nutrient concentrations, temperature, salinity and species-specific phytoplankton abundance with a high temporal sampling resolution from 2001 to 2003.

3.3.1.2 Loch Creran.

Loch Creran is 14 km long by 1 km wide (Table 3.01). It is a well flushed, two basin fjord, with shallow sill (7 m depth) at its entrance. This loch is separated into a small upper basin and a main basin, the latter with mean depth of 13 m and greatest depth of 50 metres. For further details see Tett & Wallis (1978) and Tyler (1984).

The water in Loch Creran has a typical residence time, which has been estimated to be from 7 (Tett et al. 1986) to 12 days (Tyler 1984). Salinity distributions suggest that during most of the year the loch has a two-layer estuarine circulation driven by freshwater inflow (Table 3.01). It was a site of detailed nutrient and phytoplankton study between 1972 and 1982 (Tett & Wallis 1978) but also for inorganic (Jones 1979) and organic nutrients (Tyler 1984).

In conclusion, it appears that historical data exist before the fish farm was installed in the nineties, particularly for inorganic nutrients and sometimes for

chlorophyll. However, organic nutrient data and phytoplankton data are poorly furnished.

Table 3.01. Physical characteristics of Loch Creran (after Edwards & Sharples 1991).

Parameter	Loch Creran
Volume	$183 \times 10^6 \text{ m}^3$
Sill depth	7 m
Mean depth	13 m
Greatest depth	50 m
Catchment	179 km
Relative catchment	13
Main rainfall on catchment	1.9 m.yr^{-1}
Runoff from rain	0.78 Mt.day^{-1}
Freshwater flushing	0.0043 day^{-1}
Typical tide prism	$31 \times 10^6 \text{ m}^3$
Maximum tidal exchange:	
Neap tide	0.17 day^{-1}
Mean tide	0.30 day^{-1}
Spring tide	0.43 day^{-1}
Exchange from salinity	0.16 day^{-1}

Bacterial variables (abundance and production) presented in this study are the first recorded for Loch Creran and LY1 and is one of the very few studies carried out on seasonal dynamics of bacterioplankton in temperate coastal waters (Rogerson & Laybourn Parry 1992, McManus et al. 2004, Mary et al. 2006).

3.4 Physical variables

3.4.1 Salinity, Temperature, Density.

Water temperatures ranged from 7.1 to 15.2 °C for all three stations (Fig. 3.01). Temperature reached a minimum during February and March and was warmest in August and September. There were no significant differences in water temperature

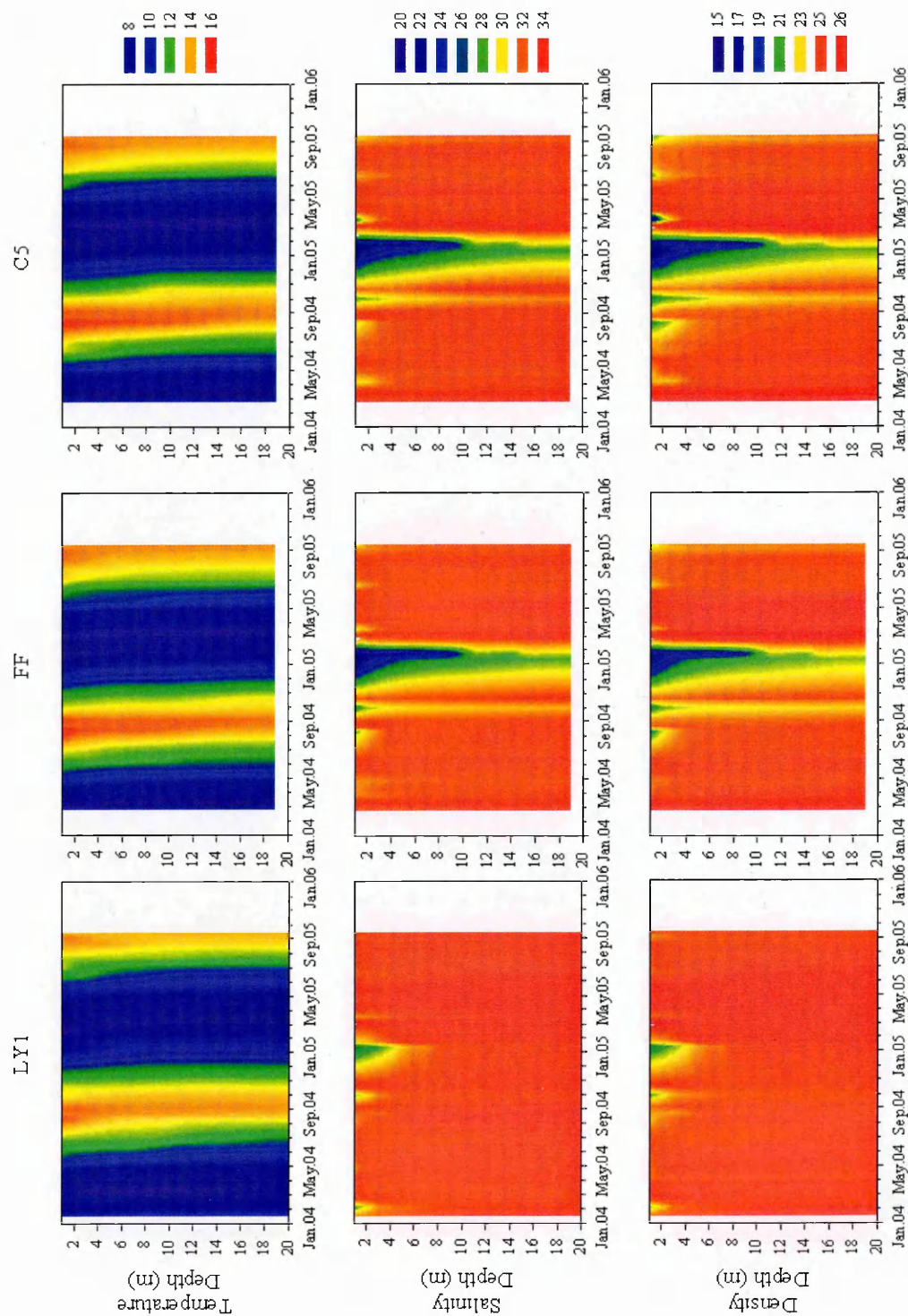


Figure 3.01. Contour plot of water temperature, salinity and density at LY1, FF and C5 between January 2004 and September 2005.

between LY1 and the two stations within the Loch (FF and C5) or between FF and C5 inside the Loch.

Salinity profiles (Fig. 3.01) showed that strong haline stratification occurred during winter months. Salinity values ranged on average from 30 to 34 at LY1 station with exceptionally low salinity events recorded at the surface on the 4th of October 2004 and 13th of January 2005 (salinities of 25 and 26, respectively).

FF and C5 exhibited a wider range of salinity (from 25.6 to 33.4) than LY1, with low salinity recorded in loch in winter between October and February. On an annual basis, salinity at 10 meters was significantly lower within the Loch (Kruskal-Wallis, p value < 0.001) than outside. Station C5 also exhibited statistically lower salinity than LY1 at 3m (Kruskal-Wallis, p value < 0.05). However, no statistical differences were found between stations within Loch Creran.

Density followed salinity profiles and ranged from 20.2 to 26.5 kg m⁻³ for all three stations (Fig. 3.01). Lowest densities were found from early October to February. LY1 exhibited significantly higher densities (Kruskal-Wallis, p value < 0.05) at 3 and 10 m than Loch Creran stations. However, no statistical differences were observed between C5 and FF.

3.4.2 Irradiance

Light penetration in the water column is shown in Fig. 3.02, as a three points running average of depth of 10% irradiance. Depth of 10% irradiance was taken as an indication of the depth under which photosynthesis may become light limited. LY1 exhibited a significantly (Kruskal-Wallis, p value < 0.01) smaller attenuation of light, with depth of 10% irradiance being, on average, 10.5 m. In contrast, FF and C5 demonstrated a similar pattern and were not statistically different from each other, had

mean depths of 8.85 m and 8.95 m respectively. Seasonal variations were difficult to detect as light attenuation may be influenced by multiple parameters such as turbidity, suspended matter or phytoplankton itself. Four noticeable events can be, however, described here: (1) low Secchi depth values in August 2004, preceded by heavy rain on the 8th, 9th and 10th, (2) low Secchi depth value in March 2005, (3) low rainfall in February 2005 and early March 2005, followed by heavy rainfall that brought the depth of 10% of light irradiance up (6 m), i.e. April 2005, and finally, (4) August 2005, which was preceded with a long period of low rainfall and weak winds.

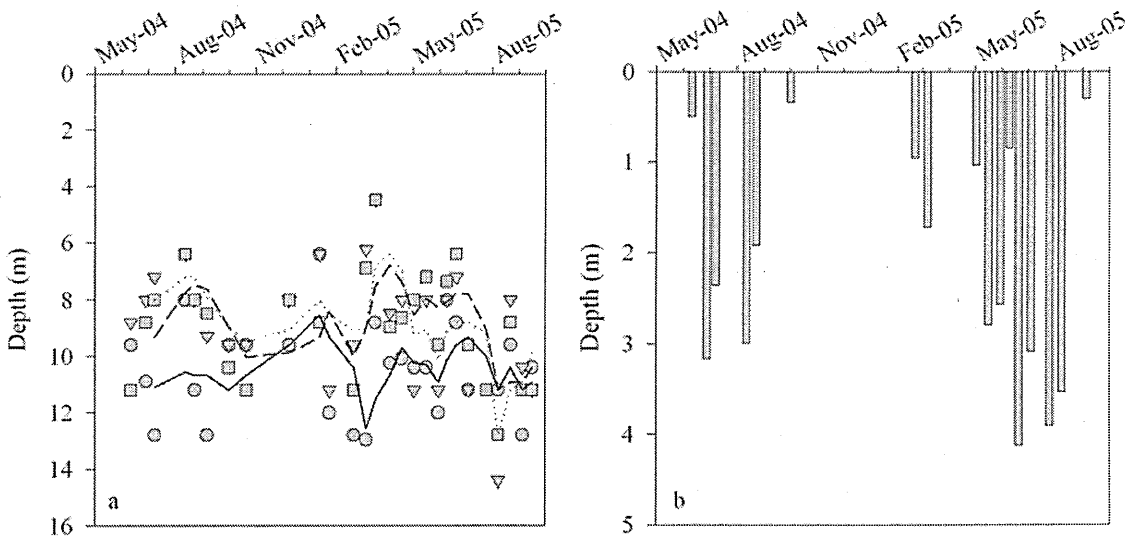


Figure 3.02. (a) Depth of 10% irradiance at LY1 (circle), FF (triangle) and C5 (square). Lines represent 3 points moving average at LY1 (solid), FF (dotted) and C5 (dashed). (b) Depth at which 50 $\mu\text{moles m}^{-2} \text{s}^{-1}$ light were available to photosynthesis.

3.4.3 Meteorological data at LY1 and Creran.

Meteorological data (average air temperature, rainfall, wind speed, and irradiance) were obtained from the Dunstaffnage weather station (56°45' N, 5°44' W) close (about 15km) to the sampling sites.

Monthly average rain between April 2004 and September 2005 ranged from 1 to 8 mm day⁻¹. Highest rainfalls were recorded in December 2004 and January 2005 and the lowest rainfalls were recorded between April and July. On a daily basis, rainfall ranged from 0 to 50 mm day⁻¹, with greater rainfall events sporadically distributed over the year. Highest rainfall events occurred on 08/08/2004, 19/09/2004 09/01/2005 and 17/08/2005 with 48.4, 40.4, 42.0 and 45.4 mm day⁻¹ respectively. The longest high rainfall event was recorded in January 2005 with six consecutive day of rainfall greater than 10 mm day⁻¹. Monthly average of wind speed varied between 5.9 and 13 knots with the strongest wind found in December 2004 and January 2005 (9.8 and 13 knots respectively). Monthly average air temperature oscillated between 5.1 °C and 15.8 °C. These temperatures varied with the season, with warmest temperatures were found in August and coldest during February 2005.

3.4.4 Inputs into Loch Creran

The contribution of fresh water to Loch Creran is on average $286.3 \times 10^6 \text{ m}^3 \text{ yr}^{-1}$ (Edwards & Sharples 1991, Austin & Inall 2002). The river Creran passes through Glen Creran and is the largest single source of fresh water into the Loch Creran system. Although there are few other minor streams, river Creran and Alt Duibhe are the major freshwater contributors to Loch Creran (Fig. 3.03). When ranked against other sea

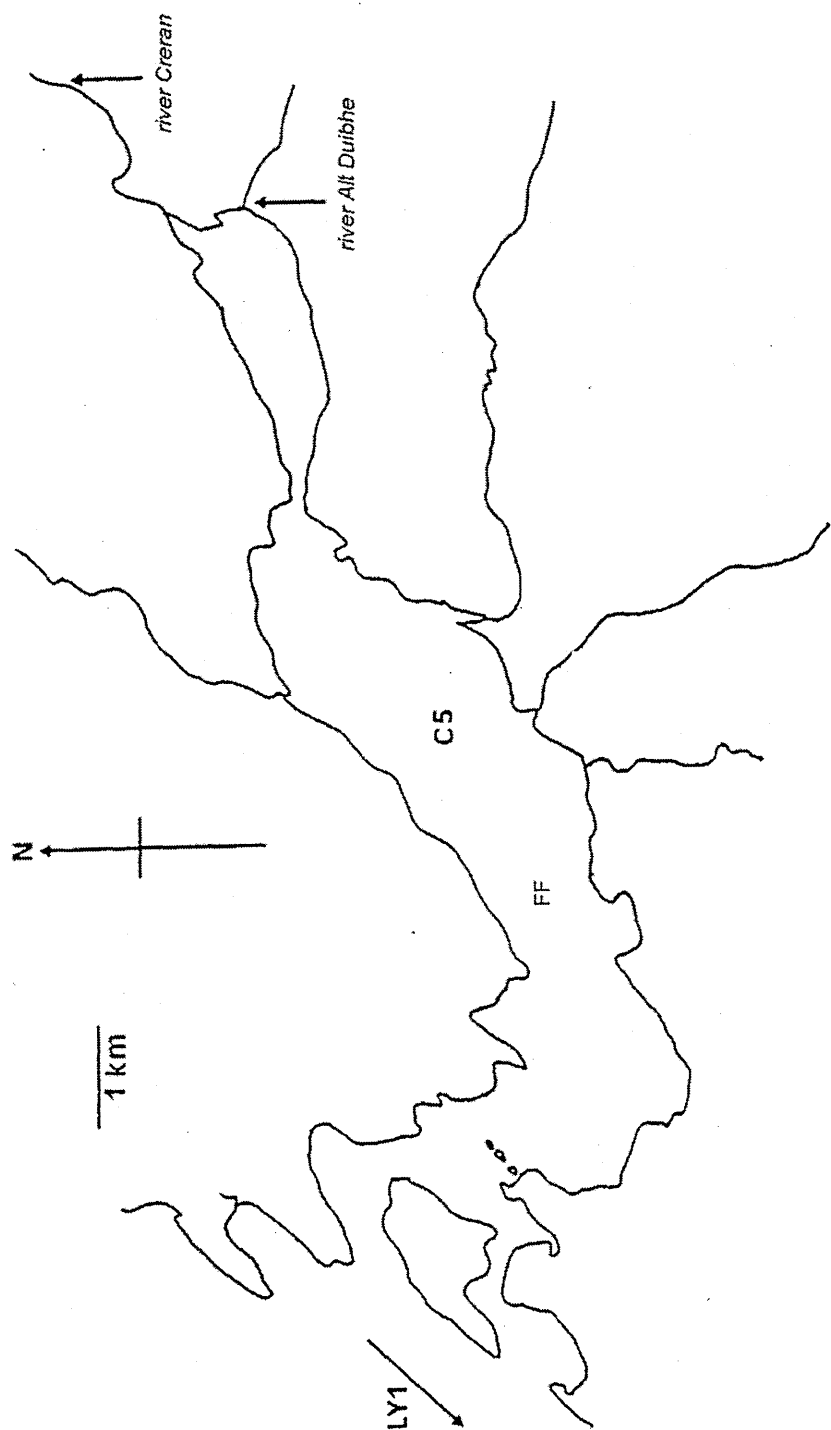


Figure 3.03. Map of principal rivers and streams of freshwater entering Loch Creran. Stations C5 and FF are indicated, as is the direction of LY1. River Creran and Alt Duibhe are designated by arrows and names.

lochs, Loch Creran has the second greatest mixing depth and the third greatest ratio of mixing depth to maximum depth.

Riverine inputs of nutrients to Loch Creran were monitored during 2005. Monthly samples were taken from river Creran and from river Alt Duibhe (Fig. 3.03), both running into the upper basin of Loch Creran. Samples were analysed for inorganic, organic nutrient concentrations and particulate organic matter concentrations, using the methods described in Chapter 2.

3.4.4.1 River flow of Creran river

River flow data were obtained from a study carried out by the HRPB/SEPA North from 1978 to 1981 and were kindly made available by SEPA Aberdeen (Fig. 3.04). The gauge was place at the mouth of the river Creran (coordinates: 56 34.17° N,

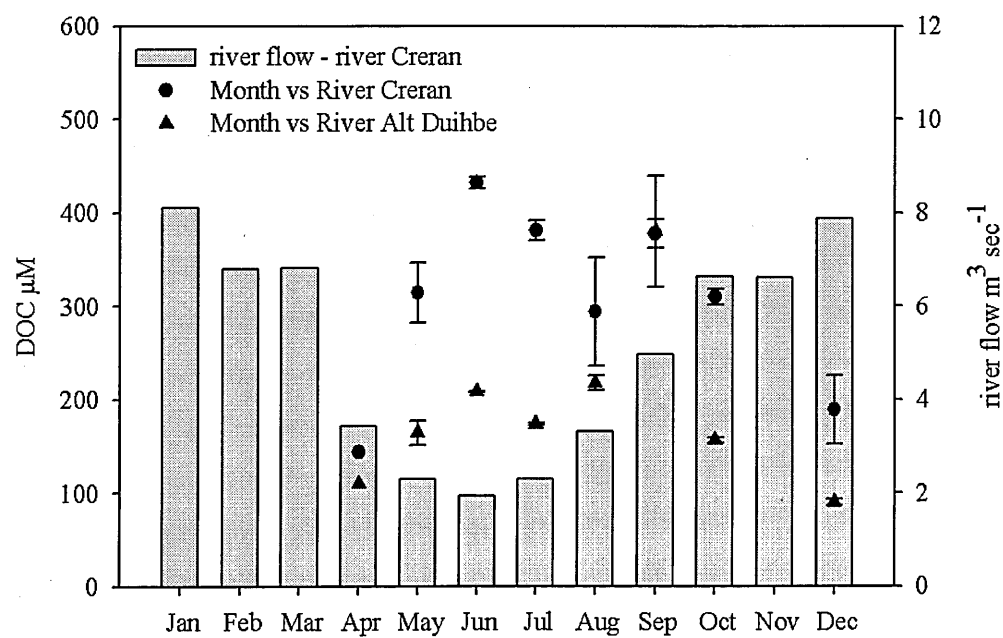


Figure 3.04. Monthly average river flow of river Creran (data average from 1978 to 1980) are represented with histograms. DOC concentrations measured during this study are represented for river Creran (dot) and river Alt Duibhe (triangle). Error bars are \pm S.E.

5 13.26° W) and daily measurements of river flow were averaged to give monthly data (Fig. 3.04). Highest flow rates ($6 - 8 \text{ m}^3 \text{ sec}^{-1}$) were found between October to March and lowest flow rates ($2 \text{ m}^3 \text{ sec}^{-1}$) were observed between May and July.

3.4.4.2 Nutrients from rivers.

Riverine inorganic nutrients entering Loch Creran are summarised in Table 3.02. Inorganic phosphorus and ammonia concentrations in both rivers ranged from 0.01 to $0.3 \text{ } \mu\text{M P}$ and from 0.06 to $2.9 \text{ } \mu\text{M NH}_4^+$. However, concentrations of silicate and nitrate covered a wider range (0.01 to $27 \text{ } \mu\text{M}$). The concentrations of nitrate in river

Table 3.02 Inorganic nutrient concentrations (μM) at the mouth of river Creran and river Alt Duibhe from April 2005 to December 2005.

	River Creran				River Alt Duibhe			
	NH_4^+	PO_4^{3-}	Si(OH)_4	NO_3^-	NH_4^+	PO_4^{3-}	Si(OH)_4	NO_3^-
25/04/05	2,4	0,3	10,0	9,4	0,6	0,1	27,7	3,3
10/05/05	0,5	0,1	0,4	6,0	0,0	0,0	17,9	2,1
26/05/05	0,4	0,1	0,0	7,4	0,3	0,0	3,5	1,0
16/06/05	0,8	0,2	0.01	2,8	0,1	0,0	3,9	0,8
21/07/05	0,4	0,1	0,1	4,0	0,1	0,1	11,4	1,0
04/08/05	3,0	0,2	2,7	5,3	0,1	0,0	6,5	0,9
19/08/05	2,4	0,3	0,0	3,6	0,8	0,2	11,9	0,9
01/09/05	0,6	0,1	0,0	2,8	0,1	0,0	5,9	0,8
15/09/05	1,7	0,1	0,1	3,7	0,2	0,0	8,7	0,7
31/10/05	0,8	0,1	0,0	3,6	1,1	0,1	11,7	1,8
06/12/05	0,6	0,0	5,8	10,8	0,1	0,0	14,2	6,2

Creran was significantly higher than the Alt Duibhe river, in all samples (average 5.4 and 1.8 μM NO_3^- for Creran and Alt Duibhe respectively). Silicates followed completely opposite trends and concentrations were greater in the Alt Duibhe river (mean 11.2 μM and 5.4 μM for Alt Duibhe and Creran respectively).

Concentrations of organic nutrients from the river (Fig. 3.04) followed an opposite trend to river flow with maximum concentrations found during June, July and September for both rivers. Maximum DOC concentrations measured in river Creran were between 350 and 420 μM , making it a potentially important contributor to organic carbon stocks in Loch Creran.

3.6 Water chemistry at the three stations

3.6.1 Inorganic nutrients concentrations

Ammonium concentrations ranged from 0 to 3 μM (Fig. 3.05) with the highest values found in September 2004, and June 2005 at all stations. Stations within Loch Creran exhibited a pronounced increase of ammonium concentrations in September 2005 that did not occur in 2004. The lowest concentrations were observed in May 2004 and 2005 and no NH_4^+ was present at all stations on three occasions (27/04/2004, 6/09/2004 and 4/10/2004). There were no significant differences between the two depths (3 and 10 m), however NH_4^+ was found to be higher at FF than C5 and LY1 (Kruskal-Wallis, $p\text{-value} < 0.05$).

Inorganic phosphorus exhibited values ranging from 0 to 0.57 μM with maximum attained generally in autumn-winter and decrease to its lowest concentrations during spring and summer months (Fig. 3.06). No significant differences in inorganic phosphorus concentrations were found between stations or depths.

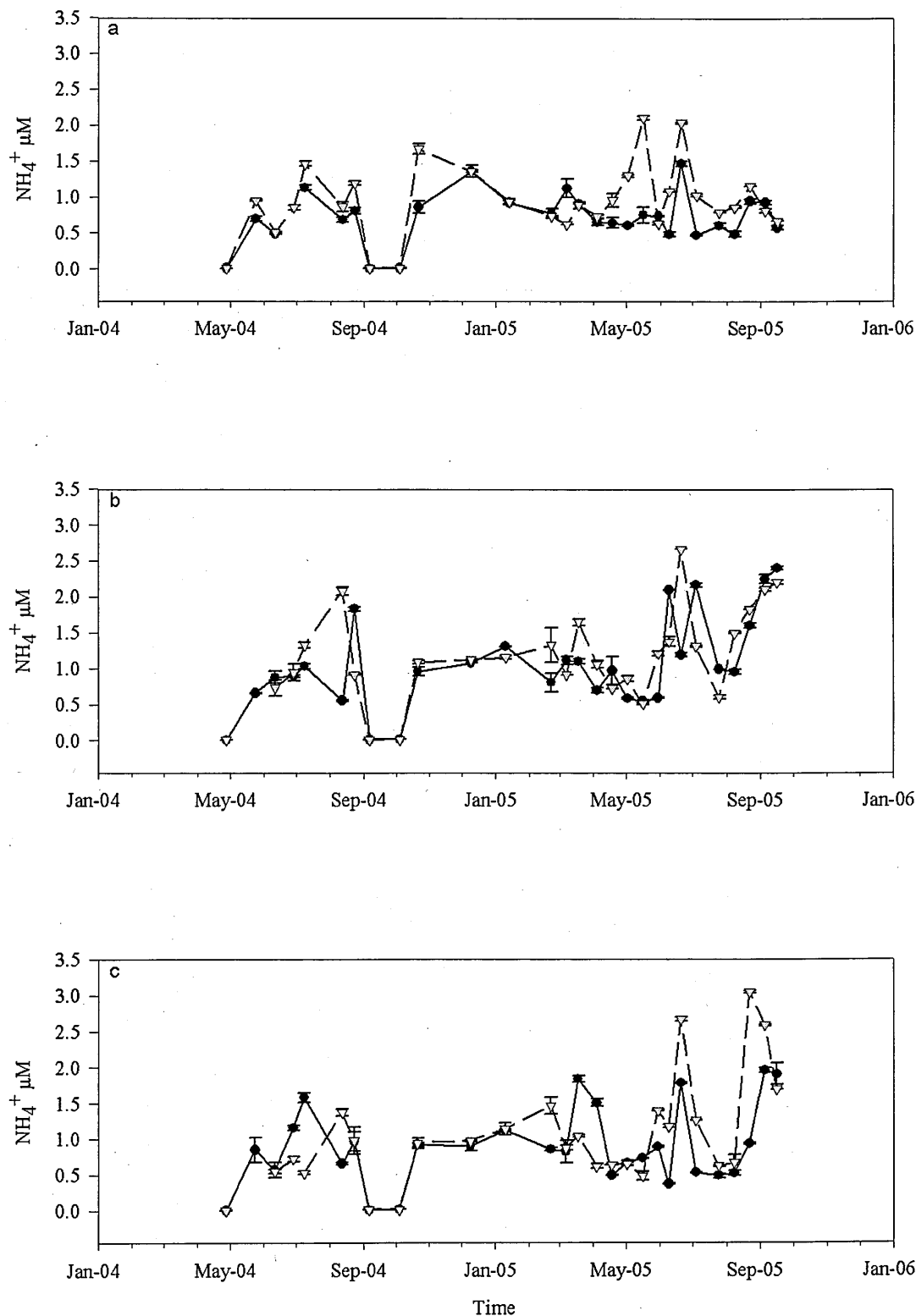


Figure 3.05. Ammonium concentrations (μM) recorded at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

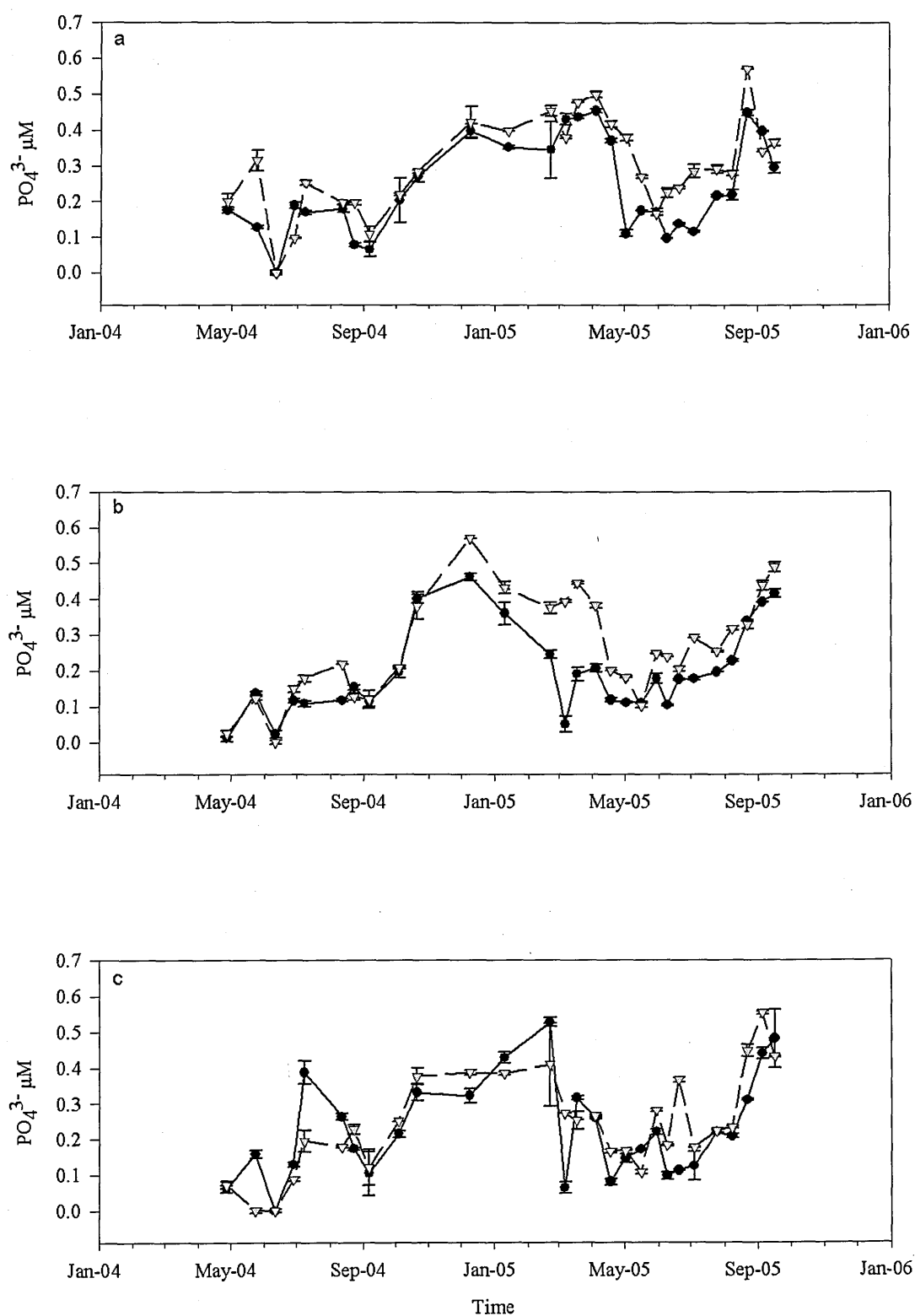


Figure 3.06. Dissolved inorganic phosphorus concentrations (μM) measured at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

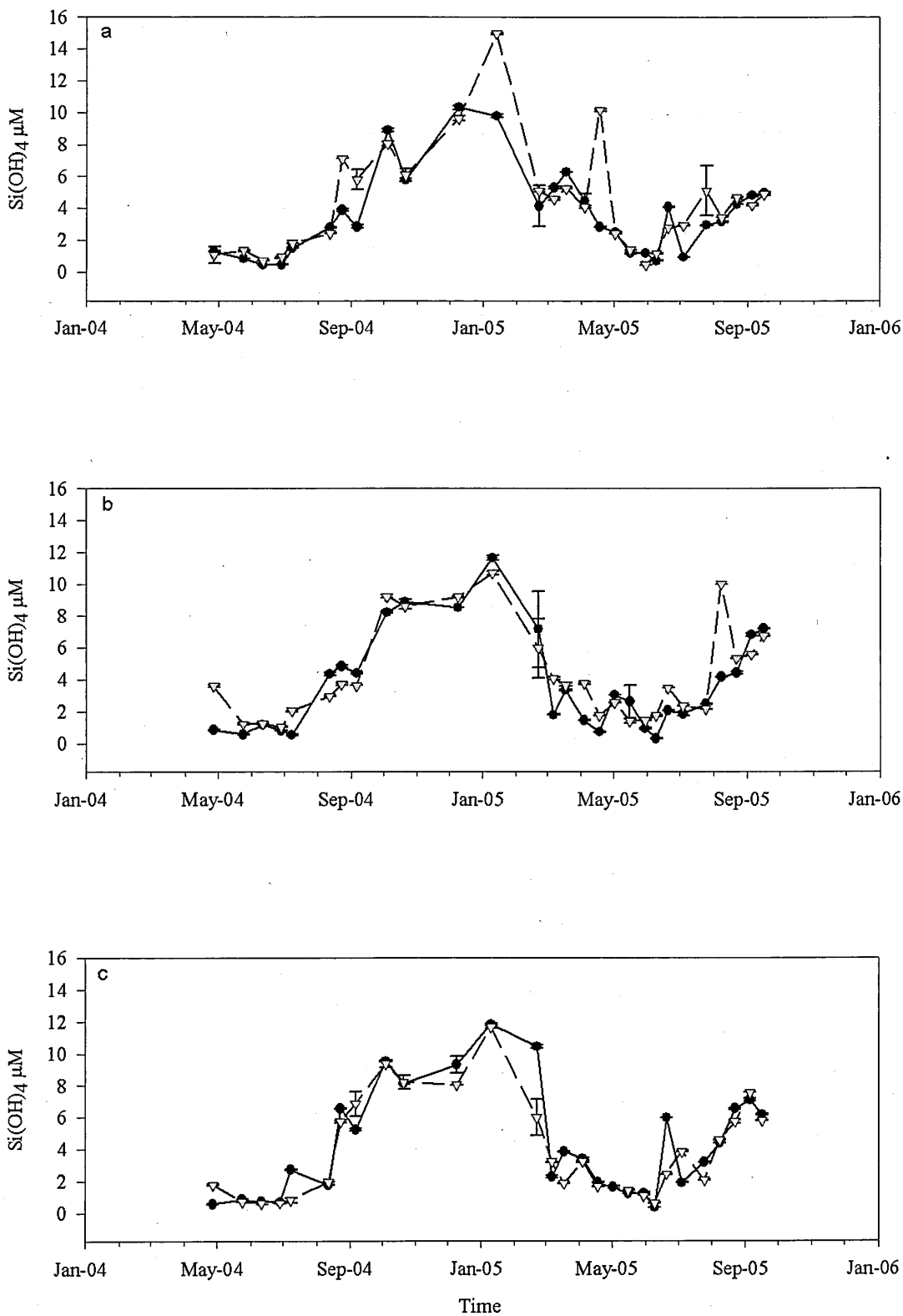


Figure 3.07. Dissolved silicate concentrations (μM) measured at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

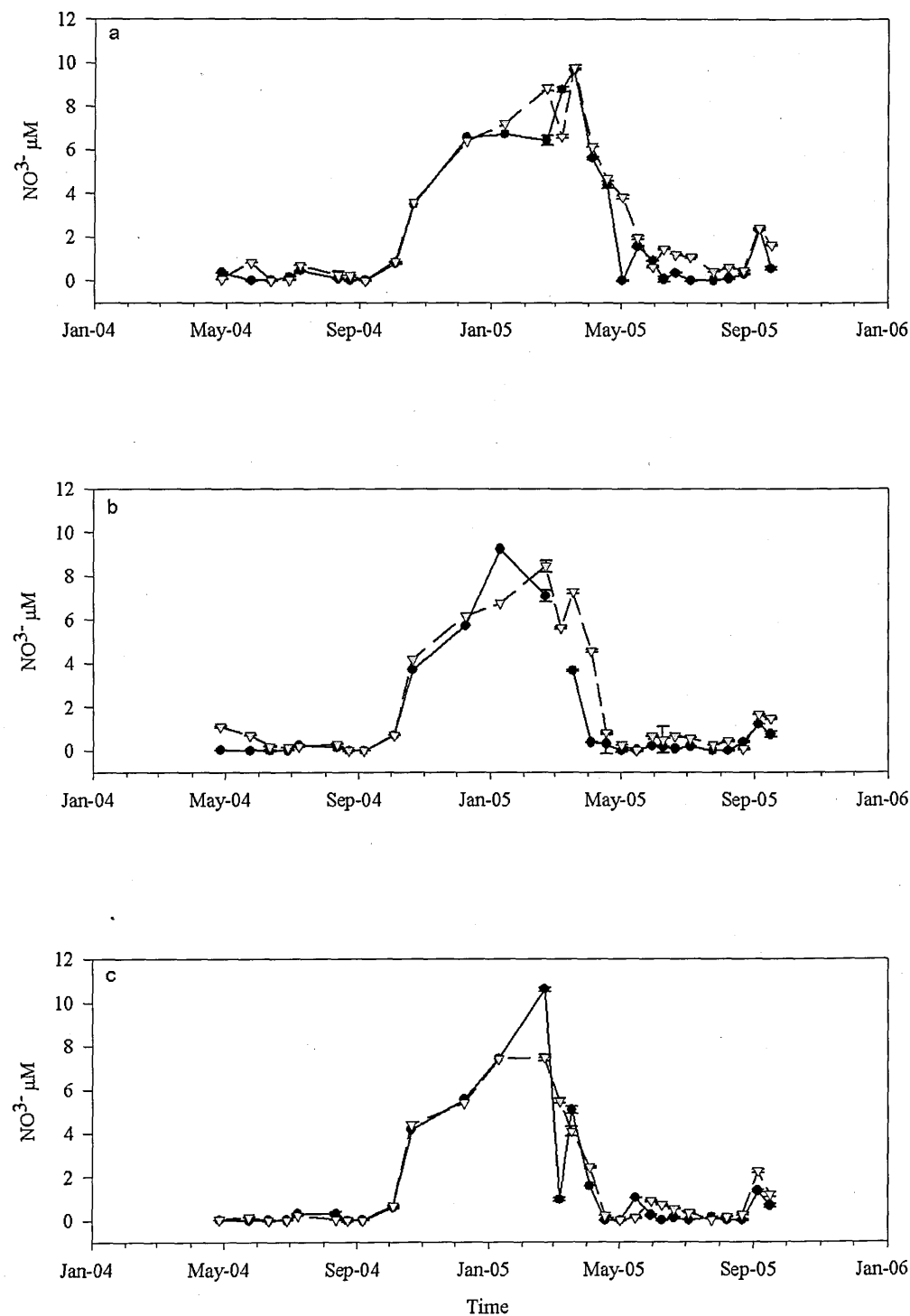


Figure 3.08. Nitrate concentrations (μM) measured at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

Silicate concentrations ranged from 0 to 10 μM and followed a similar seasonal pattern (Fig. 3.07). There were no significant differences between sites or depth at a location.

Similar to silicate, nitrate concentration varied between 0 and 10 μM . Nitrate followed the same seasonal trend as inorganic phosphorus, reaching its maximum concentrations during the autumn and winter but decreased during the spring and remained low through the summer months (Fig. 3.08). No significant differences were found between locations or depth at a location.

In this study, N to Si ratios (Fig. 3.09) calculated as total dissolved inorganic N over Si concentrations, varied with season with values above 1 found from February to May and from end of June to August. High values found during these periods suggested a potential relative lack of silicate supply to the diatom community (see Chapter 5, section 5.1).

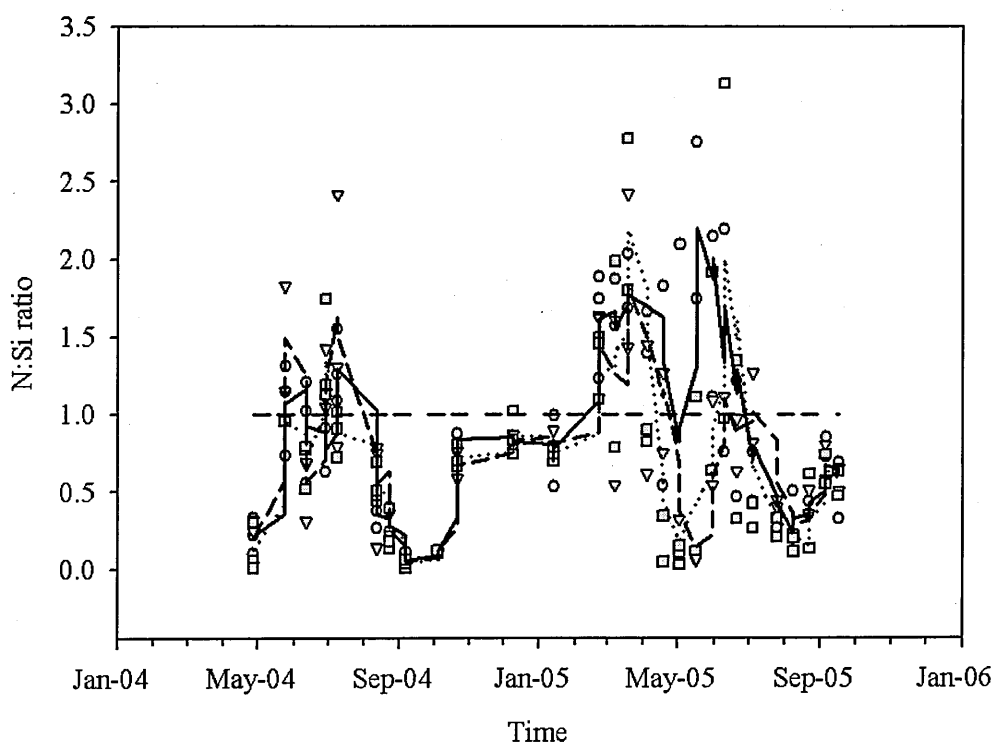


Figure 3.09. N:Si ratio calculated at LY1 (circle), FF (triangle) and C5 (square). Lines represent three points moving average at LY1 (solid), FF (dotted) and C5 (dashed).

3.6.2 Dissolved Organic Carbon and Nitrogen concentrations

DOC concentrations varied between 69 and 291 μM (Fig. 3.10). No significant differences were found either between depths or stations. However, seasonal trends were observed over the two years of the study. A marked increase in DOC concentration was observed during July and August 2004 and in 2005 between June and September.

Furthermore, increases were noticeable during the spring 2004 at stations FF and C5 and were evident for all 3 stations in spring 2005. All of these increases in DOC concentration, ranging between 100-150 μM , were followed rapidly by a decrease of similar magnitude. Finally, a winter build up of DOC was observed at the three stations, extending from late September 2004 to the end of February 2005. In addition, DOC concentrations were inversely correlated to salinity (Pearson correlation, $P < 0.01$), suggesting inputs of DOC through runoff or freshwater inputs.

DON varied from 2.2 to 41.2 μM at LY1 and from 5.7 to 30.7 μM at FF and C5 (Fig. 3.11). DON data, as calculated by subtraction of inorganic N to total dissolved N (TDN), were consequently slightly more erratic than DOC data but a few trends were still noticeable between sites. A late May/early June increase in DON concentration (20-35 μM) was observed and was greater in 2004 than in 2005. DON concentrations increased during wintertime to reach concentrations around 22 μM in January for the stations within Loch Creran, although, no significant differences were found between station or depth.

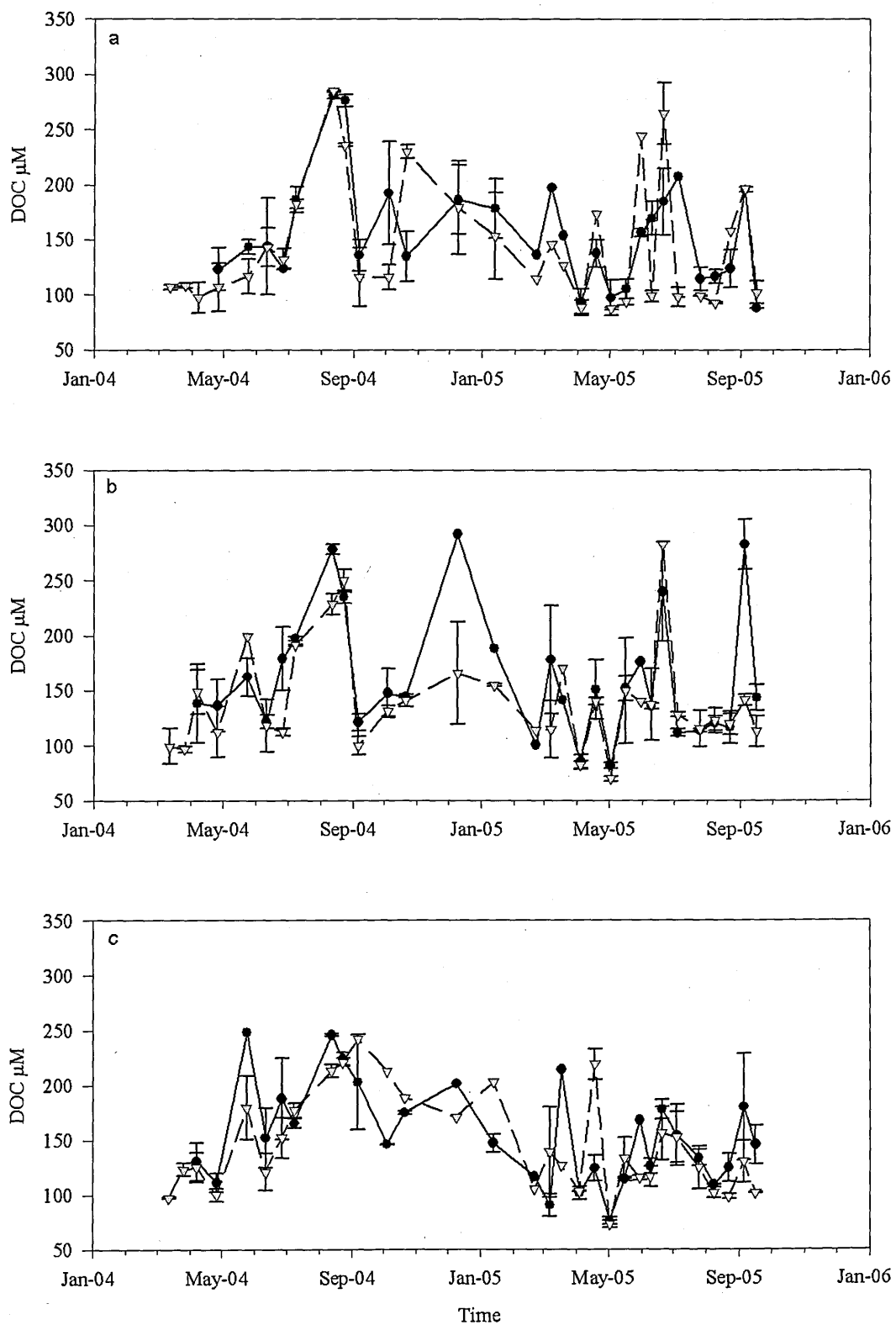


Figure 3.10. Dissolved organic carbon concentrations (μM) measured at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

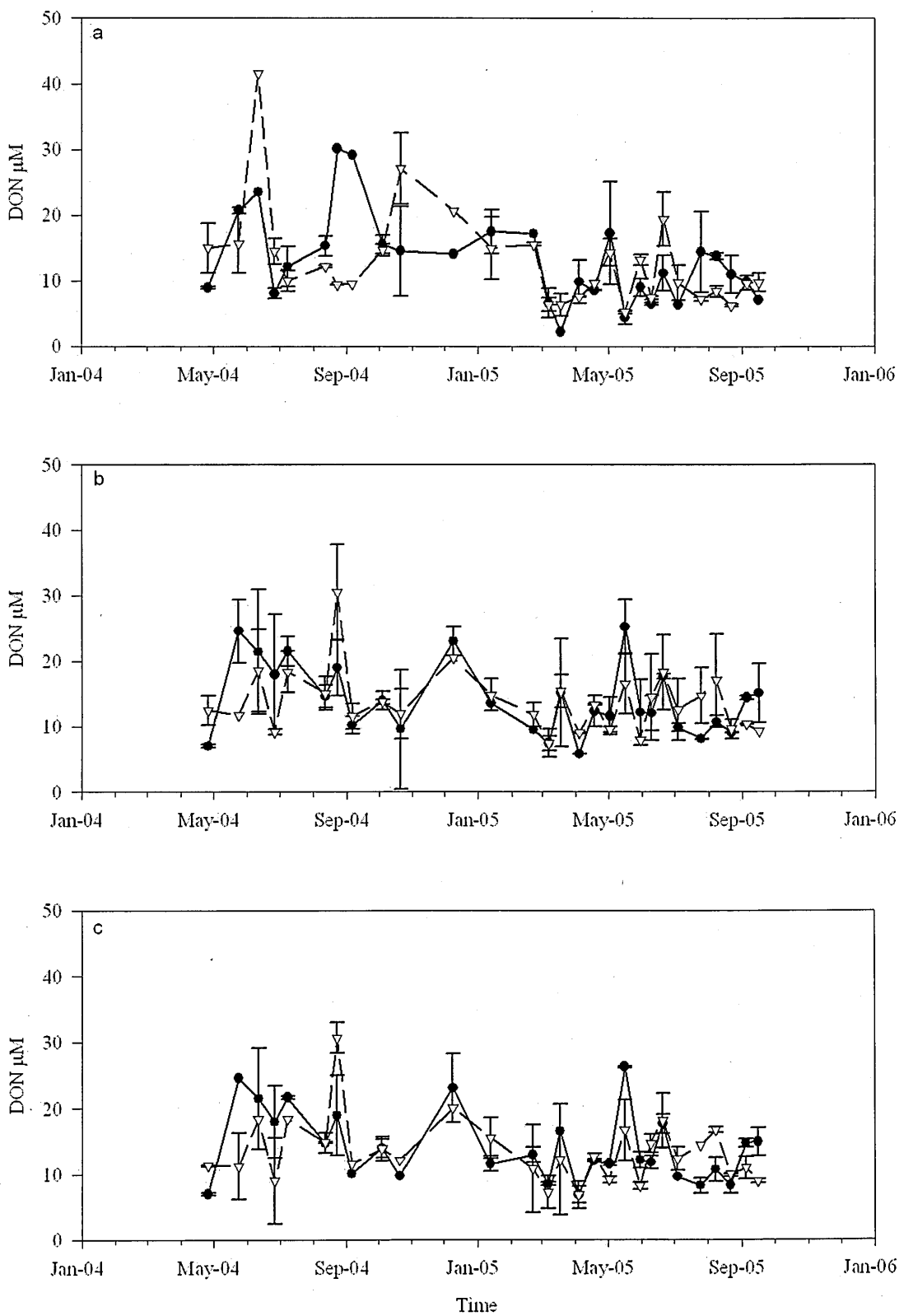


Figure 3.11. Dissolved organic nitrogen concentrations (μM) measured at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

3.6.3 Particulate Organic Carbon and Nitrogen

POC and PON exhibited similar patterns throughout the period of the study (Fig. 3.12), with increased concentration during May and late summer (August and September). These increases were greater in 2005 than 2004, with values ranging from 0.02 to 0.92 $\mu\text{gC L}^{-1}$ and 0.01 to 0.12 $\mu\text{gN L}^{-1}$. A noticeable peak was observed at three m depth at station C5 in August-September 2004 and was greater than POC or PON peaks found in 2005 (0.93 vs. 0.6 $\mu\text{gC L}^{-1}$ and 0.13 vs 0.09 $\mu\text{gN L}^{-1}$, for C and N respectively). No differences between depths were found either for POC or PON. However, POC and PON were significantly greater at C5 (Kruskal-Wallis, p-value < 0.05 and p-value < 0.01 for POC and PON respectively) than at LY1 but no differences were found between stations C5 and FF.

3.6.4 Chlorophyll *a* and Pheopigments concentrations

Chlorophyll *a* (chl *a*) and its degradation products, pheopigments (pheo) ranged from 0.08 to 10.9 mg m^{-3} chl *a* and from 0 to 0.7 mg m^{-3} pheo respectively (Fig. 3.13). Chl *a* concentrations increased between March and May and between late June to August. Spring and summer increases were more pronounced within Loch Creran in 2005. Statistical analysis demonstrated that the concentration of chl *a* was greater within the loch than at LY1 (Kruskal-Wallis, p-value < 0.05). However, no significant differences were observed between FF and C5 or between depths at these sites.

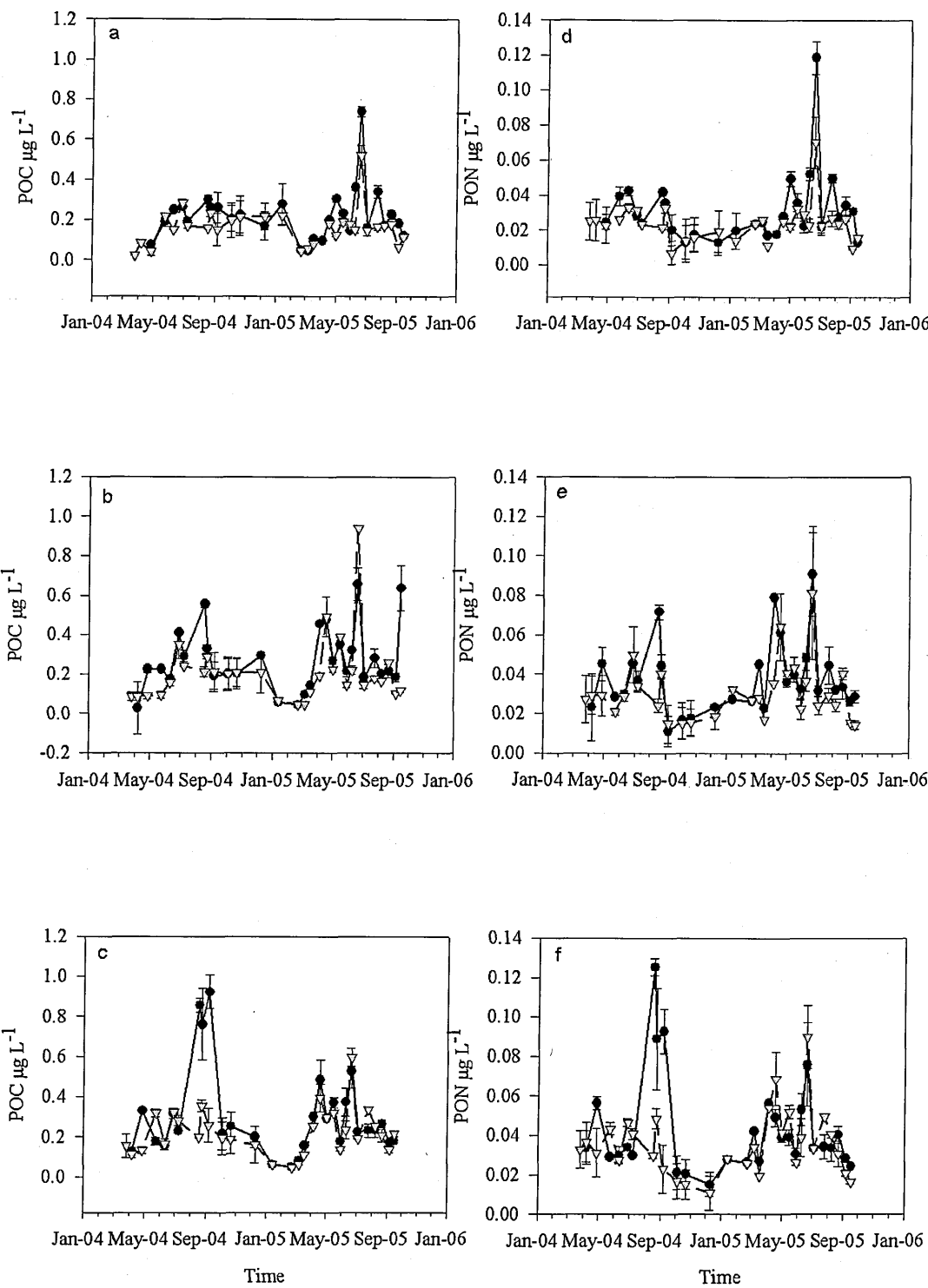


Figure 3.12. Particulate organic carbon (a, b, c) and nitrogen (d, e, f) concentrations (μM) measured at LY1 (a, d), FF (b, e) and C5 (c, f) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

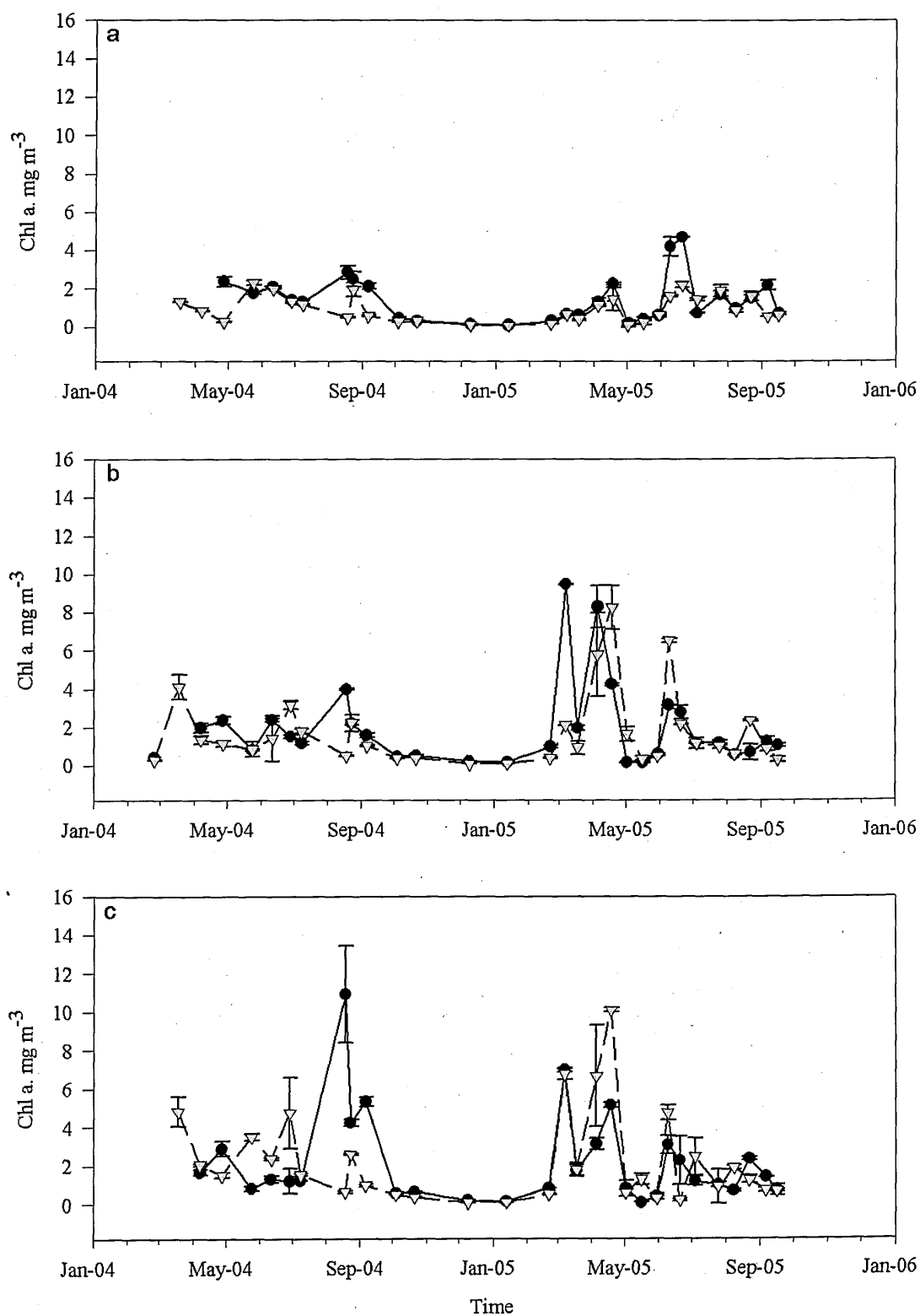


Figure 3.13. Chlorophyll a concentrations (mg Chl a m⁻³) measured at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

3.7 Biological variables

3.7.1 Marine Heterotrophic Bacteria

3.7.1.1 Bacterial Abundance

BA exhibited seasonal variations (Fig. 3.14) with values ranging from 4.4×10^8 cell L⁻¹ to 37.5×10^8 cell L⁻¹. Maxima of BA were found, at all sites, between May and September with a first increase in May - June followed by a second peak in abundance around late August - September. From late September to April, the level of BA remained low, around 1×10^9 cell L⁻¹, at all sites. The main pattern observed was lower BA (Kruskal-Wallis, p value < 0.05) at LY1 than Loch Creran. However, statistical analysis did not reveal differences between FF and C5 and no statistical difference were found at all stations over the period of the study.

3.7.1.2 Bacterial Production

BP was measured from the 12/08/2004 to the 16/9/2005. BP ranged from 0.08 to 53.2 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ with peaks of BP recorded in September 2004, March 2005, May 2005 and August 2005 (Fig. 3.15). BP exhibited very high values in September 2004 at 3 m, especially at FF and C5 (46.5 and 53.2 $\mu\text{g C L}^{-1} \text{ d}^{-1}$, respectively). Comparison of BP between stations revealed that BP at LY1 was, on average, lower than FF and C5 stations (Kruskal-Wallis, p-value < 0.01). A significant difference between depths (BP greater at 3m than 10m) was, however, found only at C5 station over the period of the study (Kruskal-Wallis, p value < 0.05).

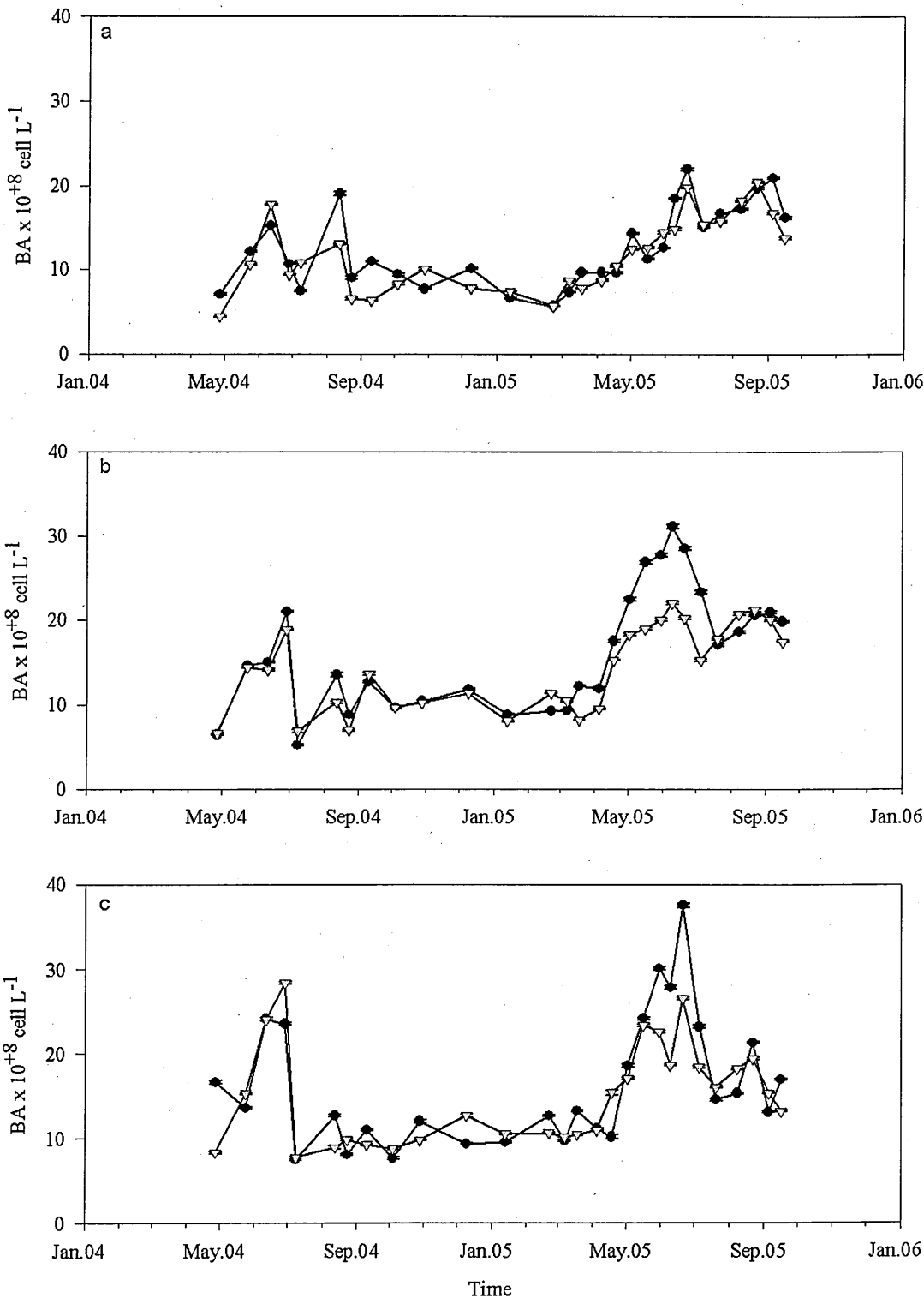


Figure 3.14. Bacterial abundance (BA) in $\times 10^8 \text{ cell L}^{-1}$ measured at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

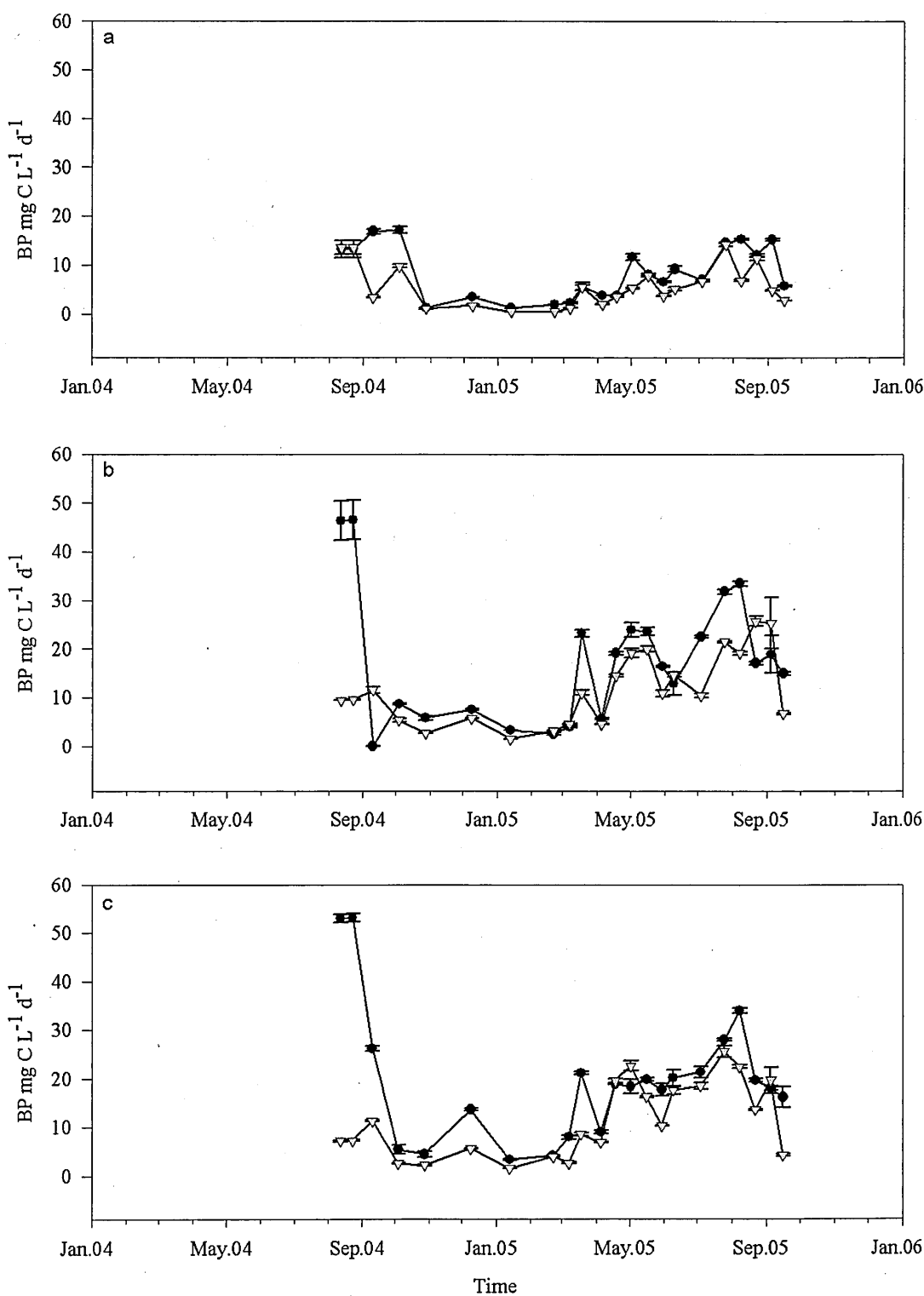


Figure 3.15. Bacterial production (BP) in $\mu\text{g C L}^{-1} \text{d}^{-1}$ measured at LY1 (a), FF (b) and C5 (c) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E. BP are calculated using literature conversion factor (cf. text).

BA and BP were positively and significantly correlated (Pearson correlation matrix, p -value < 0.01) as shown in Figure 3.16 and, when plotted against each other, BA and BP showed a linear relationship (equation of the linear regression is given in the legend of Figure 3.16).

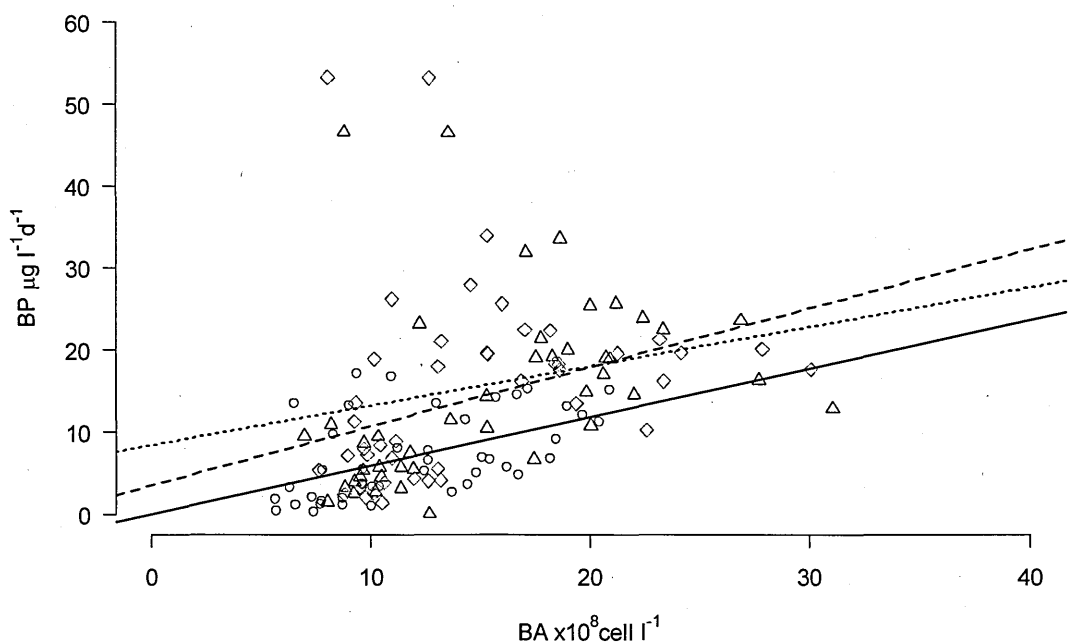


Figure 3.16. Plot of BA versus BP computed with data from LY1 (red circle), FF (blue triangle) and C5 (green diamond). Lines represent linear regressions for LY1 (solid line, $BP = 0.59 \times BA - 0.08$, $n = 60$, p -value < 0.01), FF (dashed line, $BP = 0.72 \times BA + 3.5$, $n = 60$, p -value < 0.01) and C5 (dotted lines, $BP = 0.48 \times BA + 8.4$, $n = 60$, p -value > 0.05).

3.7.1.3 Bacterial Production and Conversion Factor

An empirically determined conversion factor to relate the incorporation of thymidine to the number of cell produced from this incorporation was critical to this study to achieve its aim of comparing stations and seasonal patterns. Many studies use a literature derived conversion factors because of the logistical problems associated with determining empirical values. As conversion may be, however, time and location specific, the use of literature derived values may give erroneous results. These literature

based conversion factor, therefore, are not always relevant to the comparison of BP in different time and space scales.

3.7.1.3.1 Empirical determination of Thymidine Conversion Factor (TCF)

The results of empirical determination of TCF (experimental design described in Chapter 2, section 1.4.3) are plotted in Figure 3.17. The conversion factor experiments carried out in winter demonstrated that the increase in thymidine incorporation over time was only accompanied with a small increase in cell number. This, therefore, demonstrated little coupling between incorporation of thymidine and increase in bacterial cell number. However, it is worth noting that at this time of the year incorporation of thymidine was almost 10 times higher at FF (Fig. 3.17-d) than LY1 (Fig. 3.17-a). This trend was opposite in spring where LY1 exhibited higher thymidine incorporation rate (2 times higher than FF), while cell numbers remained in the same range at both stations. In summer, both cell numbers and thymidine incorporation rate increased exponentially. Cell numbers reached similar values ($\sim 60 \times 10^9$ cell L⁻¹) whereas incorporation of thymidine was 4 times faster at LY1 than FF (Fig. 3.17-c and f).

3.7.1.3.2 Calculations of thymidine conversion factor.

Calculations of thymidine conversion factor (CF) were carried out in three different ways using derivative, cumulative and integrative approaches, in order to assess the best usable conversion factor.

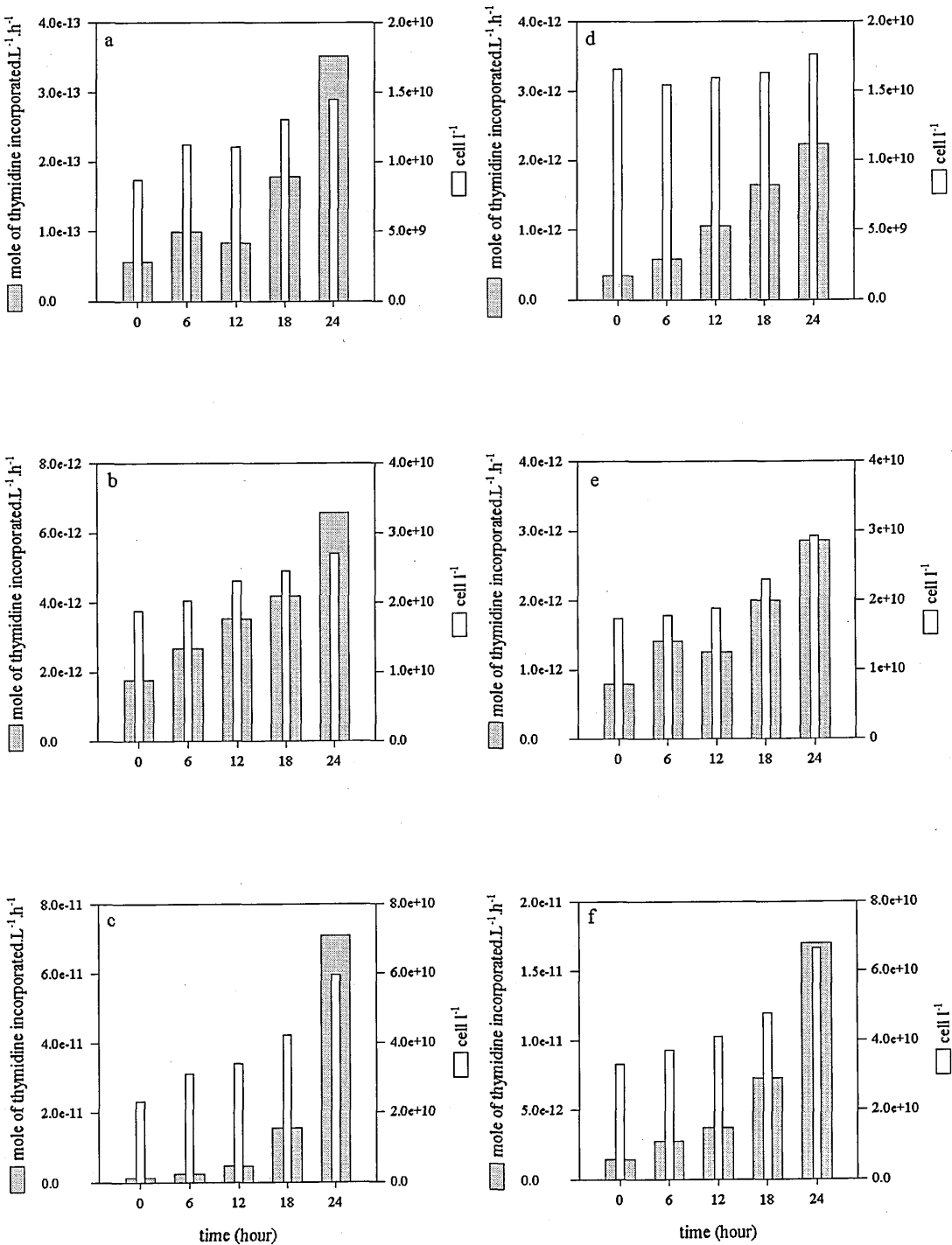


Figure 3.17. Bar chart of thymidine incorporation (gray) and BA (white) over the time course incubation (0 – 24 hours). Experiments carried out for LY1 station (a, b, c) and FF station (d, e, f) during the winter (a, d), spring (b, e) and summer (c, f). Error bars are S.E.

The modified derivative method assumes that cell numbers and rate of [*methyl*-³H]-thymidine incorporation increase similarly, that the incorporation of thymidine gives an estimate of μ , the specific growth rate, and therefore that the slope of thymidine incorporation equals μ , the slope of cell increase:

$$CF = \mu \times \frac{e^B}{e^b}, \quad \text{equation 3.01}$$

with μ : growth rate from change in abundance overtime, B: intercept of \ln (cells) against time and b: intercept of \ln (thymidine incorporation) against time.

This correspondence between cell increase and thymidine incorporation however is not always true and the slope of thymidine incorporation often exceeds specific growth rate (Ducklow & Hill 1985, Ducklow & Carlson 1992) (Fig. 3.17).

The integrative method is based on the relation between the total number of cells produced and the total amount of thymidine incorporated over time. Cell production is estimated by difference between initial and final cell counts. Total [*methyl*-³H]-thymidine incorporation is calculated by integration of thymidine uptake over time (using a quadratic fitted curve):

$$CF = \frac{[N_f - N_0]}{\int (Tdr) dt}, \quad \text{equation 3.02}$$

with N_f : final bacterial abundance, N_0 : initial bacterial abundance and $\int (Tdr dt)$: rate of thymidine incorporation integrated over the duration of the experiment. This method may lead to underestimation of TCF because the incorporation rate of thymidine (on the

denominator) may increase faster than cell production (Ducklow & Carlson 1992) (Fig. 3.17).

The cumulative method is similar to the integrative method but use the slope of the regression of cell abundance and the cumulative [*methyl*-³H]-thymidine incorporation, producing a time-weighted ratio of cells produced to [*methyl*-³H]-thymidine incorporated:

$$CF = \frac{\sum_0^i (N_i - N_0)}{\int (Tdr) dt}, \quad \text{equation 3.03}$$

Although, this method offers the best mathematical approach to TCF determination because it accounts for all the data, it is also sensitive to the lack of coupling between thymidine incorporation and cell numbers in incubated samples (Ducklow & Carlson 1992) (Fig. 3.17). These different methods provided the TCF values summarised in Table 3.03.

Table 3.03. TCF values calculated according to the three different mathematical methods.

	LY1			FF		
	Winter	Spring	Summer	Winter	Spring	Summer
Integrative	160.02	8.43	7.72	2.31	30.47	7.45
Derivative	134.03	6.66	39.05	5.59	16.94	26.18
Cumulative	nd	0.80	1.30	8.50	13.30	6.30

all values are $\times 10^{18}$ cell.mole⁻¹ of thymidine incorporated

nd: non determined

TCF exhibited large differences (up to 200 times) according to the mathematical method used. On average, the derivative method generated the largest TCF whereas the cumulative method delivered the lowest values. It is also important to note here the discrepancy in TCF calculated for winter with the three methods that demonstrate the effect of a lack of coupling between thymidine incorporation and cell numbers. No seasonal variation of TCF was obvious. However, TCF doubled from spring to summer at FF site, whereas TCF was two times greater in the spring compared to the summer at LY1.

Comparing BP calculated with literature (2×10^{18} mole thymidine incorporated cell^{-1} , Bell 1993) or empirically derived TCF (Fig. 3.18) demonstrated the importance of directly determined parameters when describing microbial processes, and to larger extent C fluxes from local to global scale. The cumulative method was preferred in this study because it accounts for all the data points of the TCF experiments and therefore gives more accurate calculation of TCF.

Visual inspection allows separating of seasons as follows:

- Summer: July, August, September and October
- Winter: November, December, January and February
- Spring: March, April May and June

This demonstrated that with a commonly used TCF (2×10^{18} cells per moles of thymidine incorporated; Bell 1993), BP at LY1 was overestimated (annual average of empirical TCF = 0.7×10^{18} cells per moles of thymidine incorporated) for each season, whereas BP at FF was always underestimated (annual average: 9.4×10^{18} cells per moles of thymidine incorporated).

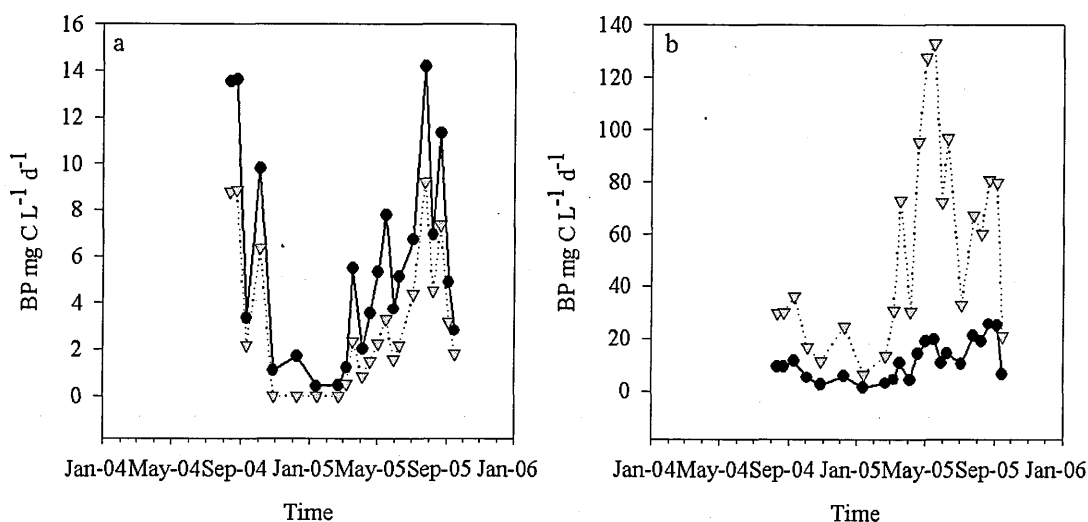


Figure 3.18. BP calculated with TCF from literature (circle) and empirically determined TCF (with cumulative method, see section 3.7.1.3.2) for this study (triangle) at LY1 (a) and FF (b).

3.7.2 Marine Nanoflagellates

Marine nanoflagellates were grouped as choanoflagellates, other heterotrophic nanoflagellates, phototrophic nanoflagellates and small heterotrophic dinoflagellates (size < 20 μm) and taxa identified (as the most common in temperate waters) are summarised in table 3.04. Although several genera were identified in each of the four groups, the results in the following figures are displayed by group in order to simplify the description of the marine nanoflagellates community.

The abundance of each of these groups varied with the season and year (Fig. 3.19). Choanoflagellates abundance peaked in May 2004, March – April 2005 and July 2005 for all three stations. In 2004, FF station exhibited a late September peak reaching 3×10^5 cells L⁻¹. Station C5 displayed the highest abundance (4×10^5 cells L⁻¹) in March 2005. Small heterotrophic dinoflagellates peaked in May-June 2004 and from May to September in 2005 at the three locations (Fig. 3.19, d, e and f). Maximal abundances

Table 3.04. Genera identified within the four groups of nanoflagellates.

Choanoflagellates	other HNAN	PNAN	Small heterotrophic dinoflagellates
<i>Bicosta spinifera</i>	Small	<i>Nitzchia</i> sp	<i>Gymnodinium</i> sp
<i>Calliacantha natans</i>	Large	<i>Navicula</i> sp	<i>Gyrodinium</i> sp
<i>Calliacantha simplex</i>	Small + flag	Other Diatom	-
<i>Cosmoeca</i> sp.	Large + flag	<i>Phaeocystis</i> sp	-
<i>Diaphanoeca</i> sp.	Pear shape	<i>Chrysochromulina</i> sp	-
<i>Parvicorbicula socialis</i>	-	<i>Cryptophyte</i> sp	-
Other	Other	Other	Other

“other” was used when identification to genus level was impossible

were around 1×10^6 cells L^{-1} and were attained in July-August 2005. Other HNAN abundance was greater in 2004 than 2005 and was maximal during August 2004 and September 2004 (Fig. 3.20, a, b and c). It is at this time that they reached their maximum abundance of $211 - 315 \times 10^5$ cells L^{-1} . A smaller increase in abundance ($20 - 50 \times 10^5$ cell L^{-1}) was recorded in 2005 around March to May.

PNAN abundances (Fig. 3.20, d, e and f) peaked in September 2004, March 2005 and September 2005 and exhibited similar abundance during both years ($130 - 200 \times 10^5$ cells L^{-1}). In 2005, an early March (7/3/2005) peak was pronounced and reached an abundance of 121, 162 and 322×10^5 cell L^{-1} for LY1, FF and C5 respectively. The PNAN, choanoflagellates and small dinoflagellates were significantly more abundant in 2005 than in 2004 (Kruskal-Wallis, p -value < 0.01). However, HNAN were significantly more abundant in 2004 (Kruskal-Wallis, $p < 0.01$). It is important to note here that data are not available before May 2004. It is therefore difficult to gain conclusive differences between years when elevated abundance of cells may have

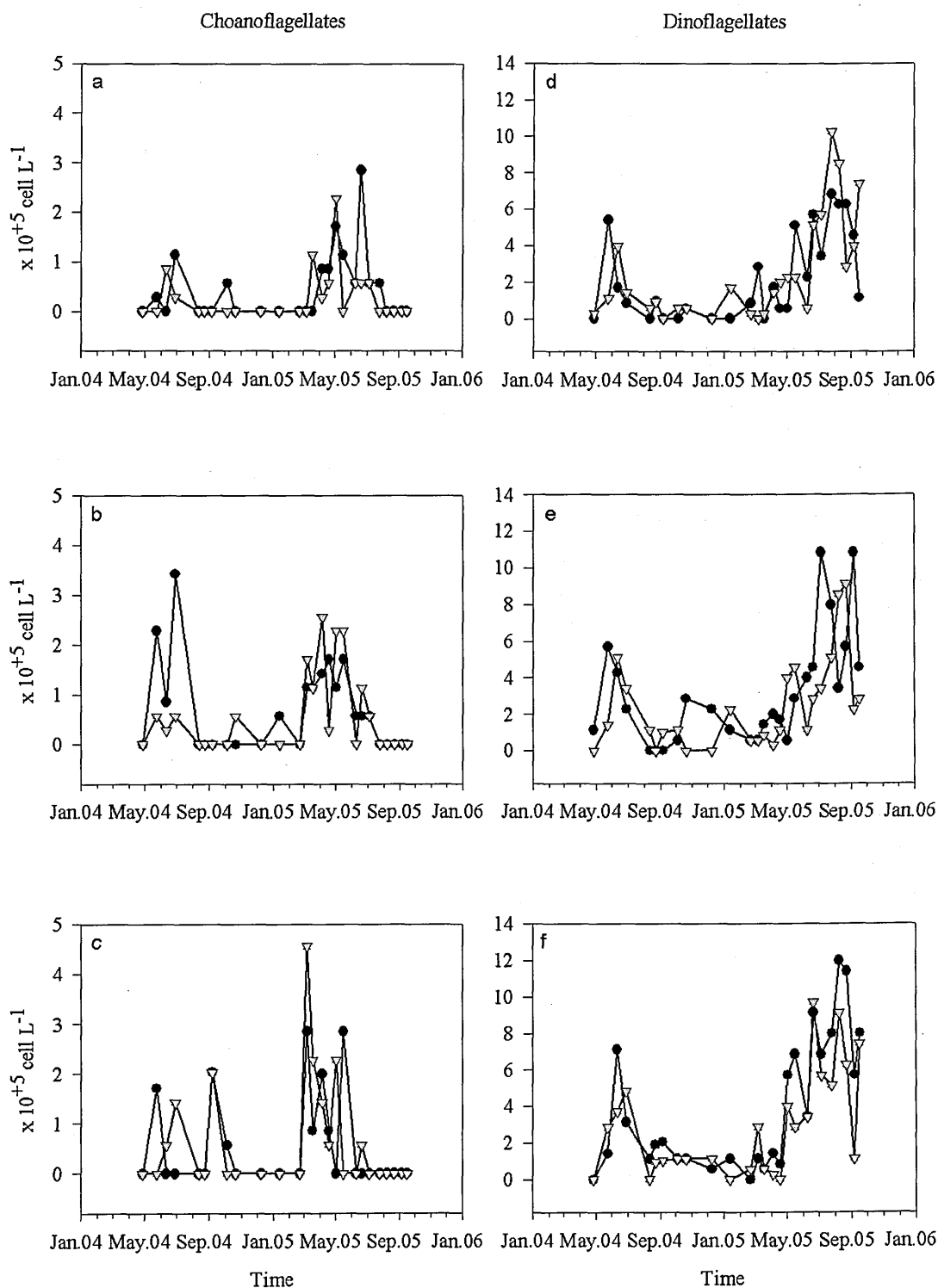


Figure 3.19 Choanoflagellates (a, b, c) and small dinoflagellates (d, e, f) abundances ($\times 10^5 \text{ cell L}^{-1}$) measured at LY1 (a, d), FF (b, e) and C5 (c, f) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

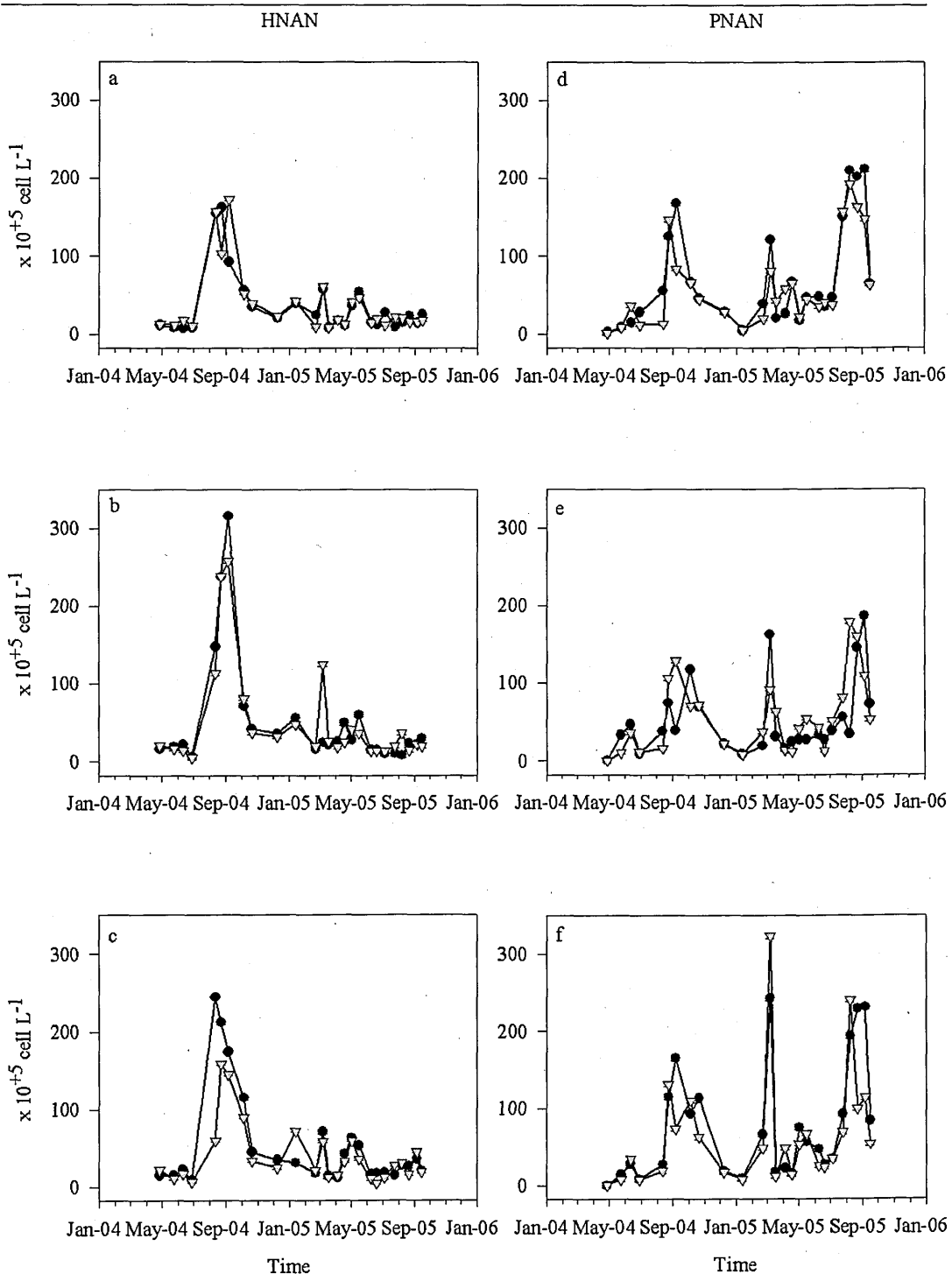


Figure 3.20. HNAN (a, b, c) and small PNAN (d, e, f) abundances ($\times 10^5 \text{ cell L}^{-1}$) measured at LY1 (a, d), FF (b, e) and C5 (c, f) at 3 m (circle) and 10 m (triangle). Error bars are \pm S.E.

occurred before spring 2004, such as for PNAN (Fig. 3.20). An alternative is to run analysis between May and September for both years, although only 2 years of data are insufficient to draw conclusions on inter-annual variability. When this is done, however, none of these four groups exhibited a significant difference either between depths at a station or between stations.

3.7.3 Relating Bacteria to HNAN grazing

HNAN was negatively correlated to BA abundance (Pearson, p -value < 0.01) and a linear relationship was found between the logarithm of their densities (Fig 3.21, see legend for equations).

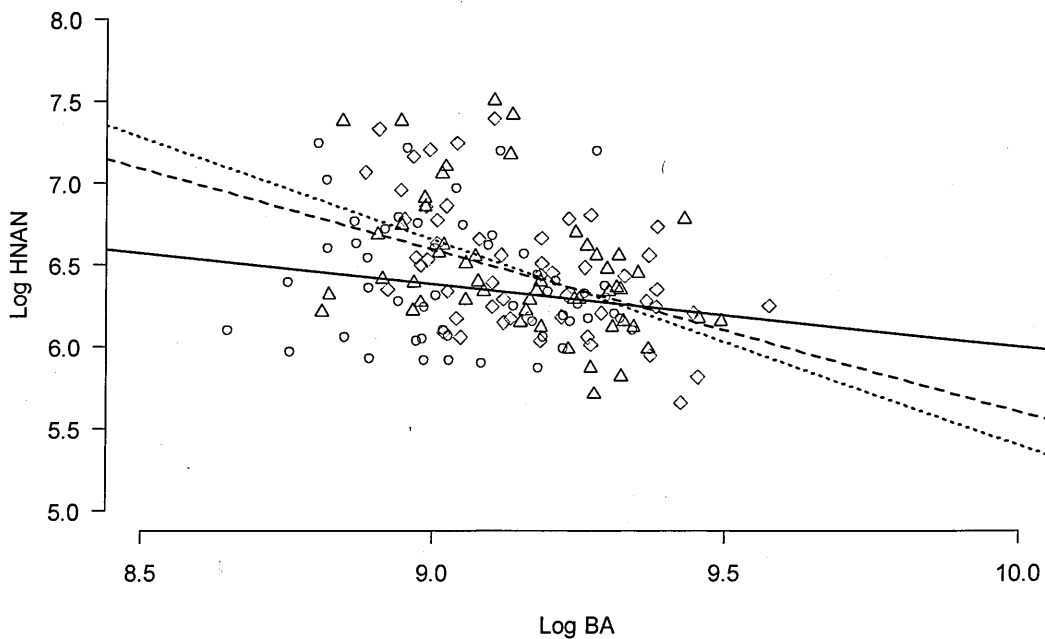


Figure 3.21. Logarithm of BA versus logarithm of HNAN abundance computed with data from LY1 (red circle), FF (blue triangle) and C5 (green diamond). Lines represent linear regressions for LY1 (solid line, $\text{Log (HNAN)} = -0.38 \times \text{Log (BA)} + 9.8$, $n = 60$, p -value > 0.05), FF (dashed line, $\text{Log (HNAN)} = -0.98 \times \text{Log (BA)} + 15.5$, $n = 60$, p -value < 0.01) and C5 (dotted lines, $\text{Log (HNAN)} = -1.24 \times \text{Log (BA)} + 17.8$, $n = 60$, p -value < 0.01).

This suggested a trophic relationship between these two populations. In order to assess the control of bacterial population and the role of HNAN as a trophic link, the logarithm of HNAN was plotted against the logarithm of BA (Fig. 3. 22), following the method of Gasol (1994). This empirical analysis allows the determination of whether bottom up (bacterial prey) or top down (grazing by higher trophic levels) controls HNAN abundance. Comparison was made with a mean realised abundance (MRA) estimated from the literature (Gasol 1994). This analysis revealed that 97% of the data fell above the MRA line (Fig. 3.22) with 7% of the data falling above the theoretical maximum (TM) of Gasol (1994). These data were therefore characteristic of HNAN control of bacterial population (or bottom up control of HNAN population).

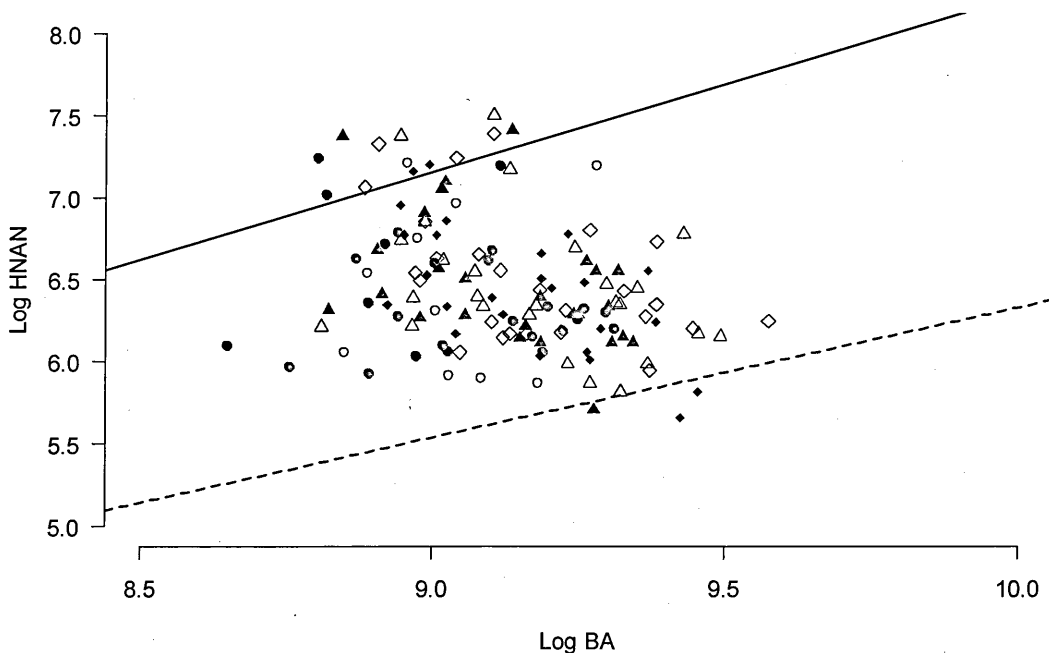


Figure 3.22. “Gasol” plot of logarithm of BA versus logarithm of HNAN abundance at LY1 (circles), FF (triangles) and C5 (diamonds) measured at 3 m (open symbols) and 10 m (filled symbols). Colors indicate data from 2004 (blue) and 2005 (red). Solid line represents the theoretical maximum (TM) and dashed line is the mean realisable abundance (MRA) calculated from Gasol (1994)

3.7.4 Phytoplankton

Phytoplankton abundance was determined monthly from April to September in 2004 and 2005 for only two stations, LY1 and FF. Phytoplankton from station C5 was not counted as other environmental variables demonstrated very little differences between C5 and FF and the taxonomic analysis was very time consuming. Figures 3.23 and 3.24 display the occurrence and relative abundance (Fig. 3.25) of each of the diatom, large dinoflagellate and ciliate taxa.

The first predominant taxa in 2004 at LY1 were *Skeletonema* sp., *Chaetoceros* sp., *Leptocylindrus* sp. and diatoms belonging to the *Pseudo-nitzschia delicatissima* group (Fig. 3.23-a). Subsequently, the diatom group of *Pseudo-nitzschia seriata* dominated the diatom community during the summer 2004, along with *Leptocylindrus* sp., the *P. delicatissima* group and *Chaetoceros* sp.

Similarly, 2005 started with the predominance of *Skeletonema* sp., but was accompanied with species of *P. delicatissima* group and *Thalassiosira* sp. During the summer of 2005, the *P. seriata* group again dominated the diatom community. In addition, *Leptocylindrus danicus* and species of the *P. delicatissima* group were common. *Skeletonema* sp and the *P. delicatissima* group persisted or even increased in number at the end of summer 2005.

In contrast, the diatom community at the FF site, within loch Creran, exhibited different predominance over the period of the study (Fig. 3.23-b). The first predominant taxa observed in 2004 were *Thalassiosira* sp. with *Chaetoceros* sp. and *Leptocylindrus minimus*. Species of the *P. seriata* group and then *Chaetoceros* sp. dominated the summer bloom in 2004. In 2005, *Leptocylindrus minimus* dominated the early summer diatom community, closely followed by the *P. seriata* then *P. delicatissima* groups. Finally, diatoms of the *P. seriata* group dominated the community in late summer.

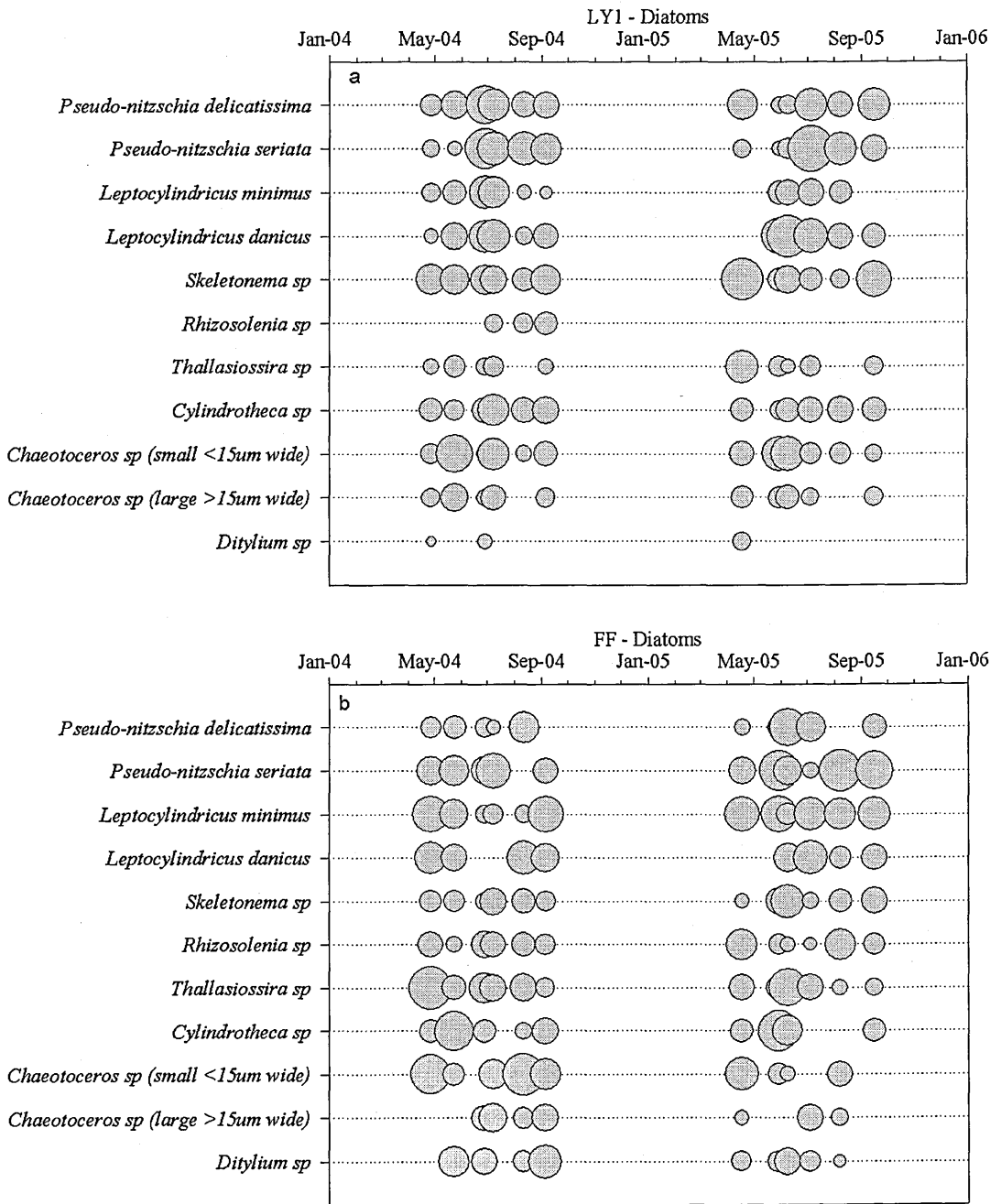


Figure 3.23. Bubble plot of diatom abundance during the period of the study at LY1 (a) and FF (b). Cell numbers were log transformed and bubbles are proportional to the abundances. Bubble sizes are directly comparable between stations.

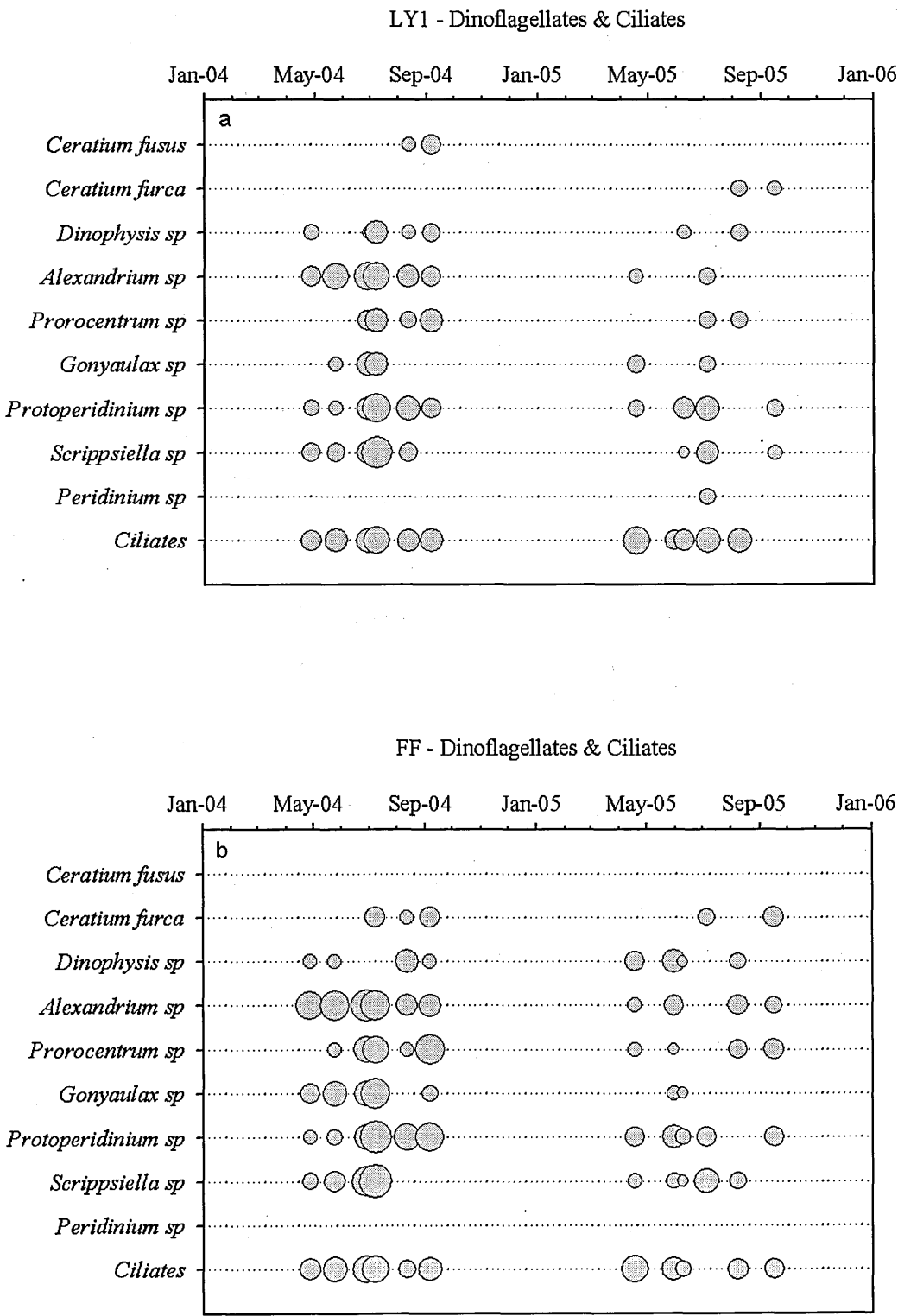


Figure 3.24. Bubble-plot of large dinoflagellate and ciliate abundance during the period of the study at LY1 (a) and FF (b). Cell numbers were log transformed and bubbles are proportional to the abundances. Bubble sizes are directly comparable between stations.

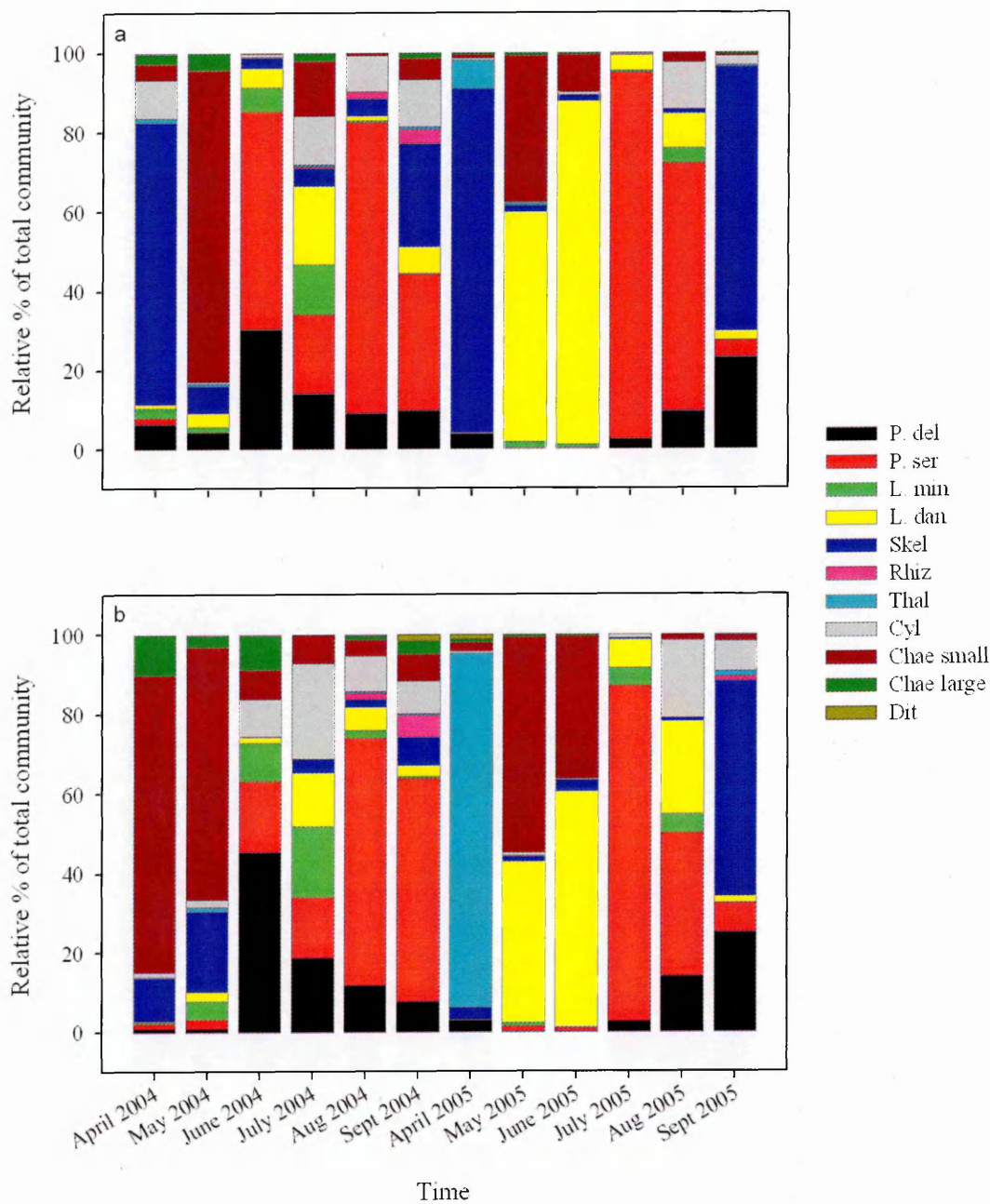


Figure 3.25. Bar chart of relative abundance to total community of diatoms for LY1 (a) and FF (b). Genus and species are given as, *Pseudo-nitzschia delicatissima* (P. del), *Pseudo-nitzschia seriata* (P. ser), *Leptocylindricus minimus* (L. min), *Leptocylindricus danicus* (L. dan), *Skeletonema* sp (Skel), *Rhizosolenia* sp (Rhiz), *Thalassiosira* sp (Thal), *Cylindrotheca* sp (Cyl), small (< 15 μ m wide) *Chaetoceros* sp (Chae small), large (> 15 μ m wide) *Chaetoceros* sp (Chae large) and *Ditylum* sp (Dit).

Dinoflagellates were prevalent in 2004 with *Alexandrium sp.* appearing first in April 2004 followed by *Scrippsiella sp.*, *Protoperidinium sp.* and *Gonyaulax sp.* *Protoperidinium sp.* persisted during late summer 2004. In 2005, the dinoflagellates community was scarce with *Protoperidinium sp.*, *Dinophysis sp.*, and *Scrippsiella sp.* being the prevalent taxa.

RDA analysis carried out at LY1 (Fig. 3.26) and at the FF site (Fig. 3.27) demonstrated that 94% and 89% (LY1 and FF respectively) of the variance (planktonic community composition) was explained by environmental data. Only significant environmental variables (Monte Carlo test, $p\text{-value} < 0.05$) are shown in Figure 3.26 and 3.27. These analyses confirmed previous observations that species occurring early in the year (*Skeletonema sp.*, *Chaetoceros sp.*, *Thalassiosira sp.*, *P. delicatissima* group, *Alexandrium sp.*) were inversely correlated with nutrients (consumption of inorganic nutrient by phytoplankton) but not correlated with temperature (remaining low in spring). However, species developing in the summer month (*P. seriata* group, *Leptocylindrus sp.*, *Protoperidinium sp.*, *Dinophysis sp.*, and *Scrippsiella sp.*) were correlated to temperature. It is important to note here the lack of influence of dissolved organic nutrients.

MDS ordination (Fig. 3.28), based on phytoplankton abundance and species composition (diatoms, dinoflagellates and ciliates); pointed out the seasonality of community composition at LY1 and FF. A seasonal progression from spring (April, May) to summer (June, July) and late summer (August, September) was recognised with the highest dissimilarity (Bray-Curtis) found between April-May and July-August with June appearing as a transition period (Fig. 3.28).

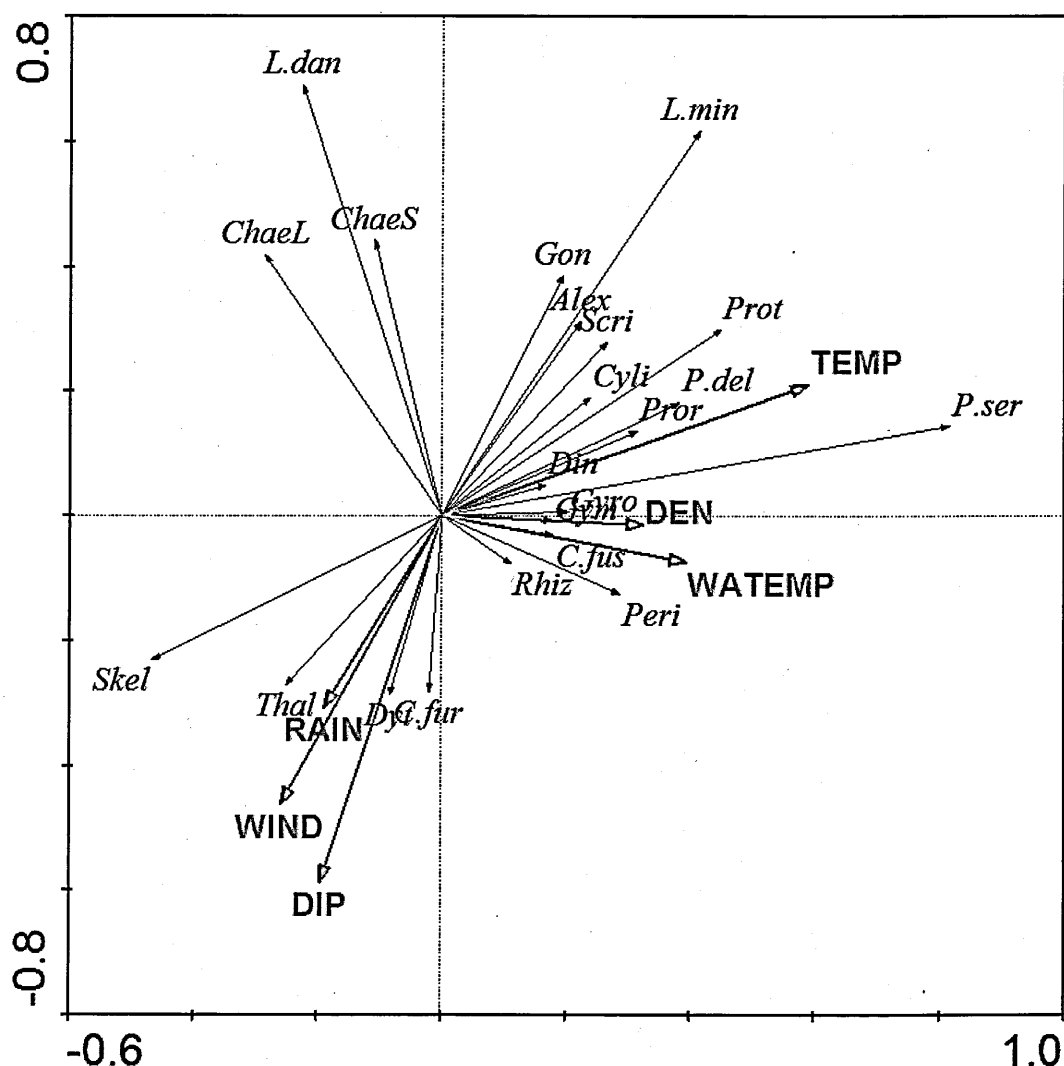


Figure 3.26. Bi-plot of RDA results for LY1. All phytoplankton species are plotted with significant (Monte Carlo test) environmental variables (TEMP: air temperature, DEN: density, WATEMP: water temperature, RAIN: rainfall, WIND: wind speed and DIP: dissolved inorganic phosphorus). Genus and species of diatoms are given as: *Pseudo-nitzschia delicatissima* (P. del), *Pseudo-nitzschia seriata* (P. ser), *Leptocylindricus minimus* (L. min), *Leptocylindricus danicus* (L. dan), *Skeletonema* sp (Skel), *Rhizosolenia* sp (Rhiz), *Thalassiosira* sp (Thal), *Cylindrotheca* sp (Cyl), small (< 15 μ m wide) *Chaetoceros* sp (ChaeS), large (> 15 μ m wide) *Chaetoceros* sp (ChaeL) and *Ditylum* sp (Dit). Genus and species of dinoflagellates are given as, *Alexandrium* sp (Alex), *Ceratium fusus* (C.fus), *Ceratium furca* (C.fur), *Dinophysis* sp (Din), *Prorocentrum* sp (Pror), *Gonyaulax* sp (Gon), *Protoperidinium* sp (Prot), *Scrippsiella* sp (Scri), *Peridinium* sp (Peri), *Gyrodinium* sp (Gyr) and *Gymnodinium* sp (Gym).

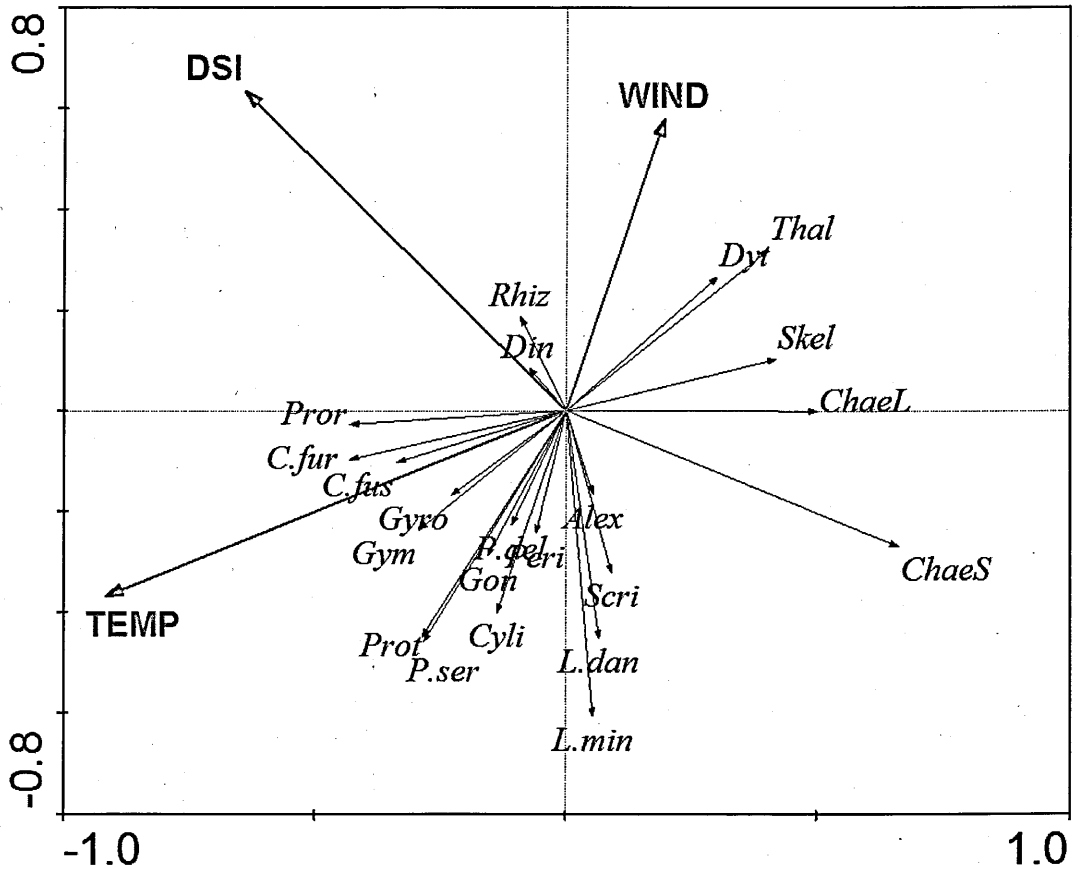


Figure 3.27. Bi-plot of RDA results for FF. All phytoplankton species are plotted with significant (Monte Carlo test) environmental variables (TEMP: air temperature, WIND: wind speed and DSI: dissolved inorganic silicate). Genus and species of diatoms are given as: *Pseudo-nitzschia delicatissima* (P. del), *Pseudo-nitzschia seriata* (P. ser), *Leptocylindricus minimus* (L. min), *Leptocylindricus danicus* (L. dan), *Skeletonema* sp (Skel), *Rhizosolenia* sp (Rhiz), *Thalassiosira* sp (Thal), *Cylindrotheca* sp (Cyl), small (< 15 μm wide) *Chaetoceros* sp (ChaeS), large (> 15 μm wide) *Chaetoceros* sp (ChaeL) and *Ditylum* sp (Dit). Genus and species of dinoflagellates are given as, *Alexandrium* sp (Alex), *Ceratium fusus* (C.fus), *Ceratium furca* (C.fur), *Dinophysis* sp (Din), *Prorocentrum* sp (Pror), *Gonyaulax* sp (Gon), *Protoperidinium* sp (Prot), *Scrippsiella* sp (Scri), *Peridinium* sp (Peri), *Gyrodinium* sp (Gyr) and *Gymnodinium* sp (Gym).

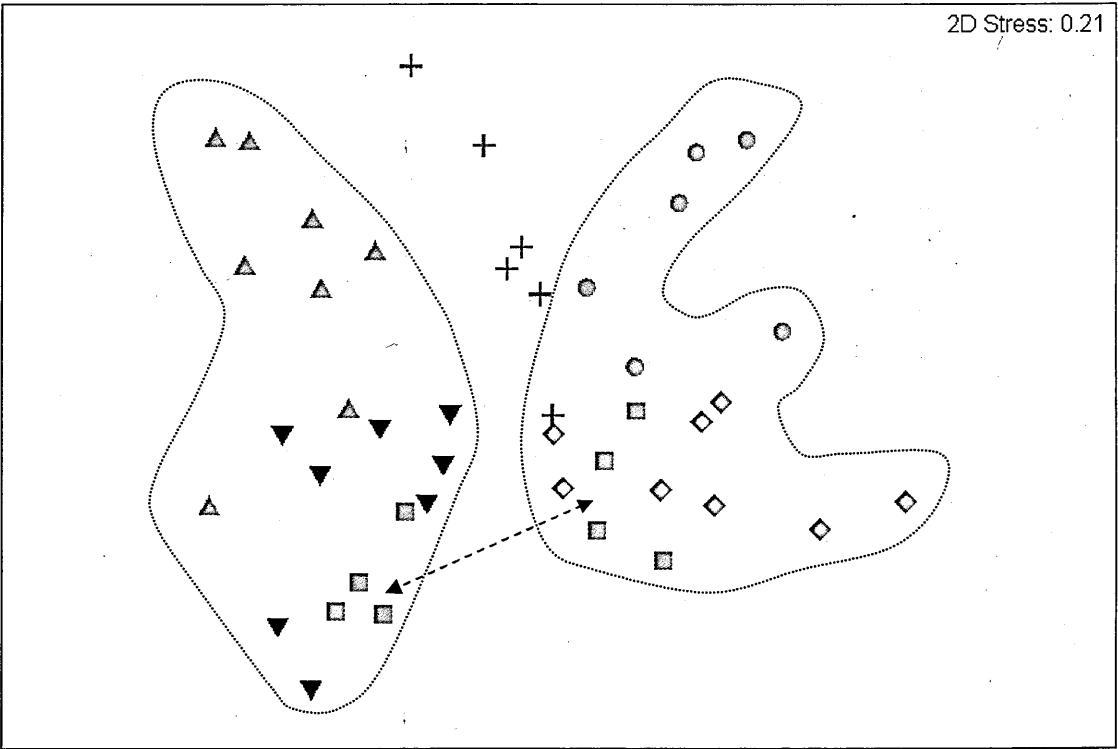


Figure 3.28. MDS ordination of phytoplankton composition. LY1 and FF data were pooled and fourth root transformed. Symbols are April (triangle up), May (triangle down), June (square), July (diamond), August (circle) and September (cross).

3.8 Summary of results

The major observations were:

- Mixing of the water column was predominant at all stations with no strong thermal stratification. However, strong sporadic freshwater input created marked haline stratification in winter, particularly within Loch Creran.
- Rivers entering Loch Creran brought significant amounts of inorganic and organic nutrients varying in form and/or concentration.
- DSi, DIP and DIN followed seasonal trends and were significantly and negatively correlated to salinity, POC, PON and Chl *a*.
- Concentrations of ammonia were significantly higher at FF than the two other sites. However, no differences in other environmental variables were found between FF and C5.
- Organic nutrient concentrations were not significantly higher at site FF than at the two other stations. However, DOC concentrations were inversely correlated with salinity, suggesting inputs of DOC through runoff or rivers.
- BA and BP were greater in Loch Creran than at LY1 but no differences were observed between C5 and FF.
- TCF varied significantly with season and site and highlighted important differences in BP. These differences were not highlighted when a single TCF value was used in calculations.
- BA was closely related to HNAN abundance, suggesting a tight control on bacterial population by HNAN grazing.
- Statistical analyses demonstrated that phytoplanktonic communities were seasonally different and the variability in phytoplankton assemblage composition could be partially explained by environmental parameters.

3.9 Discussion

3.9.1 Sources of nutrients

3.9.1.1 Nutrients from mixing

Physical parameters and inorganic nutrient concentrations measured at each site demonstrated a typical winter mixing of the water column (demonstrated by the negative correlation between water temperature and density, Pearson, $p < 0.001$) bringing up nutrient (demonstrated by the negative correlation between inorganic nutrient, N, P and Si and water temperature) and building nutrient stocks to be used by plankton during the spring bloom. These stations are characterised by a well-mixed water column with haline and/or thermo-haline stratification (Tett & Wallis 1978, Solórzano & Ehrlich 1979). Coldest waters occurred mostly in March and the warmest in August. Temperature increases from spring to summer and decreases during the winter as expected in coastal waters. These stations are characterised by a shallow layer of brackish waters at the surface (1 to 4 m depth) mainly occurring during autumn and winter when rainfall is greatest. Below this brackish layer salinity is higher but sometime strongly influenced by freshwater, as revealed by the abrupt diminution of salinity and density (Fig. 3.01). These observations were similar to those of Tett et al. (1978) for Loch Creran. Highest salinity was found usually between May and early June (Grantham 1983b, c, Fehling 2004). Due to flushing by water from the Firth of Lorne driven by a fjordic estuarine circulation, Creran's maximum nutrient levels would thus be expected to be close to those of the Firth (Jones 1979). This may explain the similar nutrient levels observed between the Firth of Lorn (LY1) and Loch Creran.

Both Loch Creran and LY1 are influenced by freshwater inputs as witnessed by the negative correlations (Pearson, $p < 0.001$) of inorganic nutrients (DSi, DIN and DIP) with salinity at both depths. This observation is in agreement with previous studies

(Grantham 1983b, c, Fehling 2004). High freshwater input events were highlighted by the density profiles that showed the extent to which the water column was affected. The fact that the salinity and density at LY1 was not as much affected as in Loch Creran might be explained by a higher flushing of the water body at LY1 (open coastal station) than in Loch Creran. This also confirmed the more exchange-restricted conditions within Loch Creran.

3.9.1.2 Nutrients from rivers

River Creran brings, on average, $160 \times 10^6 \text{ m}^3 \text{ year}^{-1}$ of freshwater to Loch Creran, which represents about half of total freshwater received by Loch Creran over a year and makes river Creran the main contributor of freshwater to Loch Creran. Therefore, nutrients brought by river Creran and, to a lesser extent, smaller rivers, such as the Alt Duibhe, may be considered as the major allochthonous nutrient sources to the microbial community in Loch Creran (see also section 3.9.1.3). In terms of organic carbon, the two rivers may supply about $3.2 \text{ g C m}^{-3} \text{ year}^{-1}$ (based on DOC measurement in g C m^{-3} multiplied by the annual average of freshwater input from river Creran) to the loch. Based on TDN measurements, N inputs from rivers reach $0.22 \text{ g N m}^{-3} \text{ year}^{-1}$. The riverine origin of DOC proposed here was confirmed by negative correlation (Pearson, $P < 0.001$) between DOC concentrations and both salinity and density.

In terms of inorganic nutrients, river Creran and Alt Duibhe contributed differently to the form of nutrient transported into Loch Creran. River Creran appeared to be a major N supplier whereas, Alt Duibhe, had higher concentrations of silicates. The type of land traversed by the rivers may explain these differences in the form of inorganic nutrients. The contributions of rivers to Loch Creran and, to a certain extent

LY1, inorganic nutrient concentrations were highlighted by negative correlation between nutrient concentration and salinity (and density; see section, 3.9.1.1).

3.9.1.3 Nutrients from the Fish farm

One of the original objectives of this work was to determine whether a point source input of nutrients from the fish farm may result in enhanced concentrations of inorganic and/or organic nutrients close to the fish farm station compared to i) the further station C5 within Loch Creran or ii) the Firth of Lorn station, LY1. One might hypothesise that these enhanced inputs of nutrient would be expected to provoke a subsequent increase in autotrophic and heterotrophic biomass and productivity.

Although there was no evidence that organic nutrient concentrations were elevated at FF, an estimation of the contribution of fish farm activities to the organic nutrient stock of Loch Creran is possible. Hall et al. (1990) estimated that 878 – 952 kg C t⁻¹ of fish produced and 95 – 102 kg N t⁻¹ fish produced were lost to the environment. The same authors estimated that, among these losses, about 49% of C and 48% N were lost as solute releases (e.g total environmental loss minus sedimentation). Assuming a consented production 1500 tonnes over a two years cycle for the fish farm in Loch Creran, estimates of organic C and N contribution from the fish farm to the whole loch (total volume) are therefore 1.84 g C m⁻³ y⁻¹ and 0.19 g N m⁻³ y⁻¹, respectively. These estimates are approximately two orders of magnitude smaller than rivers contributions of dissolved organic material to Loch Creran. It hence confirms the predominant role of rivers as sources of “new” DOM to Loch Creran.

Statistical analyses revealed that concentrations of both DOC and DON were not different (Kruskal-Wallis, $p > 0.05$) at FF compared to the two other stations. This, therefore, refutes the *a priori* ethos of enhanced concentrations of organic nutrients

from FF and raises the question of what controls the level of dissolved organic nutrients in Loch Creran? (see section 3.9.1.4)

However, while DOM concentrations were not elevated, another important input of FF in terms of nutrients was the significant increase in the concentration of ammonia compared to C5 or LY1. This feature has been demonstrated in numerous studies (Hall et al. 1990, Wu 1995, Arzul et al. 1996) as NH_4^+ is the main form of N released in fish excreta (see section 3.9.2.3, see also Chapter 4).

3.9.1.4 Nutrients generated by the microbial community

One cannot neglect the role played by the marine microbial community in the production of inorganic and organic nutrients. Because of the difficulties in assessing such processes, these were not directly measured in this field study. However, Chapters 5 and 6 give indirect estimates of N uptake, N regeneration and DOM production by phytoplankton for samples from LY1 and Loch Creran.

Primary producers are mainly consumers of inorganic nutrient (Dugdale & Goering 1967) and this was corroborated by the negative correlation (Pearson, $p < 0.05$) between inorganic N, P and Si with chl a , POC and PON, as indicators of biomass. In the open ocean, DOM is mainly produced by primary producers (Larsson & Hagstrom 1979, Lancelot & Billen 1984). However, in coastal and estuarine environments, DOM sources are various and hence autochthonous sources might not be the predominant origin of DOC and DON (Cauwet et al. 2002).

It is important to note here that limitation of phytoplankton growth by nutrients (see Chapter 1 for details) was likely to occur at LY1 and in Loch Creran as suggested by the N:Si ratio (Fig. 3.09). This inorganic nutrient limitation of either N or Si, depending on the season, provided the motivation to conduct the experiment described

in Chapter 5 and is further discussed in the same chapter. If we include the results on the production of organic C and N by phytoplankton from the experiment detailed in Chapter 5 we can summarised the contributions of the various sources of organic nutrients to Loch Creran (Table 3.05). This highlights the major contributions of phytoplankton in terms of organic nutrient supply to the loch, followed by rivers and finally the “theoretical” contribution of the fish farm. Assuming an annual average bacterial production of $10.1 \text{ mg C m}^{-3} \text{ d}^{-1}$ (data from C5 at 10m), that all sources supply biologically labile DOC and that the BGE in costal waters is 0.27 (del Giorgio & Cole 2000), we can estimate the BCD of the bacterioplankton and hence the C turn-over time in Loch Creran (Table 3.05). These values gives insights into the contributions of the different sources, such as the very short turn-over time of the DOM originated by the fish farm, which could explain the absence of enhanced DOM concentrations (see section 3.9.1.3). This also highlights the overall short turn-over time of all these DOM sources, suggesting a highly dynamic environment in loch Creran.

Table 3.05. Various sources of organic C and N for Loch Creran and carbon turnover time for each of these sources.

	DOC (mg C m^{-3})	DON (mg N m^{-3})	BCD ^a	C turnover
Rivers	3.2	0.2	37.4	0.08 days ^b
Fish farm	1.8	0.2	37.4	0.05 days ^b
Phytoplankton	46.0	6.7	37.4	1.23 days ^b
Total	51	7.1	37.4	1.36 days

^aBCD ($\text{in mg C m}^{-3} \text{ d}^{-1}$) calculated from equation 1.03 with BGE and BP given in the text above.

^bturn-over time calculated assuming that the source was the unique source of DOC.

3.9.2 Microbial community dynamics

3.9.2.1 Bacterial production and dissolved organic matter

DOC concentrations range from 50 to 1500 μM in coastal and estuarine environments (Hansell & Carlson 2002) and hence cover the range of values found in this work (73 – 291 μM). Similarly, DON concentrations (2.2 – 41.5 μM) fell in the range of values observed in comparable regions of the marine environment (9 – 54 μM) (Bronk et al. 1998, Hansell & Carlson 2002).

In coastal waters, as in this study, the DOM pool is highly dynamic due to the variety of its sources, and the characteristically strong mixing regime and the elevated biological activity (Cauwet et al. 2002). BP values fell within the range found in other studies of coastal, estuarine or fjordic environments (Table 3.06). The data in Table 3.06 show that BP is also highly variable, ranging from zero to almost 200 $\text{mg C m}^{-3} \text{ d}^{-1}$. In this study, BP varied with the season and with site (Fig. 3.15).

In addition, conversion factors involved in the determination of BP in aquatic systems have a major effect on calculated values of BP, as shown in this study. It is therefore important to take of variation in conversion factors in account. Overall TCF values range from 0.1×10^{18} to 60×10^{18} cell mole⁻¹ of thymidine incorporated (Ducklow & Carlson 1992) and $0.2 - 2.3 \times 10^{18}$ cell mole⁻¹ in fjord and coastal area (Table 3.06) with exceptional values reaching 52×10^{18} cell mole⁻¹. TCF found at LY1 and within Loch Creran were within the range of values observed in similar ecosystems (Table 3.06). Although a conversion factor might greatly affect the BP value, it is important to have standardised values that can be used in comparative studies (Ducklow & Carlson 1992, Fukuda et al. 1998).

Table 3.06. Bacterial production (BP), thymidine conversion factor (TCF) and bacterial production to primary production ratio (BP:PP) in fjordic and coastal studies (update from Ducklow and Carlson 1992).

Location	Method	BP (mgC m ⁻³ d ⁻¹)	TCF 10 ¹⁸ cells.mole ⁻¹	BP:PP (%)	References
Fjords					
Saanich Inlet	TDR	7.2 - 71	0.2 - 1.3	10	Fuhrman & Azam (1980)
Howe sound, B.C.	TDR	0 - 36.9	1.2	9 - 189	Albright & McCrae (1987)
Mobile Bay	TDR	14 - 179	2	-	McManus et al. (2004)
Loch Creran	TDR	0 - 130	6.3 - 13.3	-	This thesis
Coastal Ocean					
Georges Bank	TDR	4.5 - 11	4.7	-	Caron et al. (2000)
Scripps pier	TDR	0.7 - 53	0.2 - 1.3	>10	Fuhrman et al. (1980)
Georgia Bight	TDR	5 - 192	1.3	25	Newell & Fallon (1982)
Southern Baltic Sea	LEU	0.26 - 106.3	-	-	Feuerpfeil et al (2004)
North Sea	TDR	12 - 52	1.4 - 52	44 - 68	Lancelot & Billen (1984)
Oosterschelde	TDR	0.5 - 7	2.1	5	Laanbroeck et al. (1985)
Irish Sea	FDC	15 - 90	-	-	Turley & Lochte (1985)
Gulf of Finland	TDR	3.2 - 38.9	0.9 - 4.7	-	Tuomi (1997)
North Sea	TDR	5.3 - 11.9	1.1	-	Rosenberg et al. (1990)
Oosterschelde	TDR	15 - 90	-	-	Laanbroeck & Verplanke (1986)
Lynn of Lorne, LY1	TDR	0 - 50	0.8 - 1.3	-	This thesis
TDR: thymidine incorporation method, LEU: leucine incorporation method, FDC: frequency of dividing cell method					

3.9.2.2 Conversion factors

Incorporation rate of tracer is compared to bacterial biomass (or abundance) over time. The main advantage of this technique is that conversion factors are calculated with natural assemblages of bacteria. TCF is measured for the particular ecosystem studied and it allows a “correction” of theoretical factors. The critical assumption is that there is no difference in conversion factor (or rate of incorporation) compared to an undisturbed sample. A theoretical standard conversion factor of $0.4 - 0.5 \times 10^{18}$ cells mole⁻¹ of thymidine incorporated has been calculated assuming i) a 25 mol% of thymidine in DNA, ii) a bacterial DNA content of 2 – 4 fg cell⁻¹, and iii) no de novo synthesis or exogenous dilution. Estimates derived from different methods vary in their principle (incorporation into DNA, protein, observation of cell division). Using a higher final concentration of ³H thymidine can reduce variability of conversion factors, as a lack of uptake saturation occurs at low concentrations of ³H-thymidine. Furthermore, Tuomi (1997) argued that an increase of incorporation of thymidine into other macromolecules (different to DNA) might result in an underestimation of TCF and therefore BP. This uncoupling between incorporation of thymidine and increase of BA was observed in this study during the winter and might have explained the reason of elevated or incalculable (infinite value) TCFs. Although ³H-thymidine incorporation is a controversial method (reviewed by Bell 1990, Robarts & Zohari 1993), over half of published bacterial production data are derived using this method (Ducklow, 2000).

3.9.2.3 Bottom-up control of bacterial population

Heterotrophic bacteria have been recognised as the major consumers and remineralisers of organic matter in the ocean (Pomeroy 1974, LeB Williams 1981, Fuhrman & Azam 1982). Although the coastal ocean is a highly productive region in

terms of phytoplankton, it is difficult to draw the same conclusion regarding the productivity of heterotrophic bacteria and the DOM dynamics (Ducklow 2000).

This study suggested that available DOM was taken up rapidly in the loch to support the higher BP found at FF and C5 compared to LY1. In fact, there were no significant differences in DOC or DON concentrations between LY1 and Loch Creran. Furthermore, DOC and DON were not directly correlated to BA ($p = 0.335$ and $p = 0.941$ for DOC and DON respectively) or BP ($p = 0.087$ and $p = 0.105$ for DOC and DON respectively).

Annual primary production (PP) was not assessed in this study; however, Tyler (1984) estimated the total gross annual PP of Loch Creran to be 54.7 g C m^{-2} , based on ^{14}C incorporation. In this study, depth integrated average BP for loch Creran was $162 \text{ mg C m}^{-2} \text{ d}^{-1}$, assuming a mean water column of 15 meters. This gives an annual BP of 49.4 g C m^{-2} over the period of the present work. Therefore, this calculation suggested that BP would represent approximately 90 % of Loch Creran PP. This further suggests that bacteria were tightly coupled to PP and could have removed a significant proportion of this PP. Numerous studies have highlighted the importance of extracellular releases as a source of DOM from phytoplankton (see Chapter 5, Nagata 2000). The percentage of extracellular release (PER: percentage of PP released as DOC) might reached up to 80 % of PP in some cases, such as during the declining phase of phytoplankton bloom (Lancelot & Billen 1984), and high PER values have been attributed to the release of large amount of C-rich (e.g carbohydrates) DOM compounds (Mykkestad 1995, Benner et al. 1997, Granum et al. 2002). These compounds might potentially support a significant part of the bacterial carbon demand (Amon & Benner 1994, Norrman et al. 1995).

The relationship between bacteria and C stock was further corroborated by the positive correlation between BP and POC (Pearson correlation, $p < 0.001$). The

correlation between BP and POC highlighted an important contribution of bacterial C to organic carbon (3.2 – 78.5 %), especially at low POC concentrations, which was, on average, 24 % of POC (Fig. 3.29). Results indicated that chl *a*, an indicator of phytoplankton biomass, was also correlated to BP (Pearson correlation, $p < 0.001$) and was higher within Loch Creran than at LY1 (see 3.6.4, this Chapter). This further corroborates the relationship between phytoplankton production and bacterial activity, suggesting that the DOM produced by phytoplankton in Loch Creran might have supported the higher BP observed within the loch. However, the calculated coupling between BP and PP (BP:PP = 90%) cannot only be explained by the sole presence of PER that supply energy for bacteria. This would thus suggest that bacteria may required more nutrient and therefore that the bacterial carbon demand (BCD) was supplemented by other sources of DOC in Loch Creran.

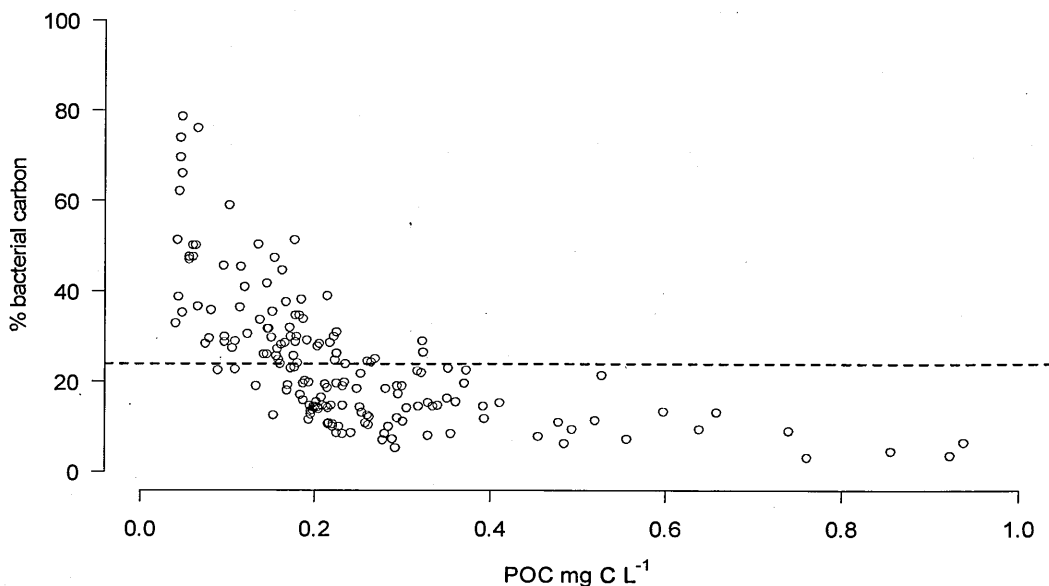


Figure 3.29. Relationship between % of bacterial carbon and the POC concentration. Data pooled for the three stations over the time of the study period. Dashed line represents the calculated average contribution of bacterial carbon to total POC (i.e. 24%).

It was demonstrated above (section 3.9.1.2, this chapter) that rivers and small streams entering Loch Creran supply DOM. The availability of such DOM to biological utilisation (Rolff & Elmgren 2000), however, is still a matter of controversy (Hedges et al. 1997, Cauwet et al. 2002). Hedges et al. (1994) suggested that riverine DOM is 70 % HMW, while the HMW pool is only 30 % of surface ocean DOM, therefore indicating that it must be partly labile. Similarly, HMW DOM is often considered as biologically refractory, Ogawa (1999) reported that HMW DOM composition is close to biologically known molecules (e.g. proteins, lipids) whereas LMW is more complex and poorly determined and this would suggest a more bioavailable character of HMW DOM. The paradigm of greater bioavailability of HMW DOM has also been suggested (Amon & Benner 1996). More recently, Seitzinger et al. (2002) suggested that 23 to 30 % of TDN from terrigenous origins was available to biological consumption as DON. This characteristic of riverine DOM might have furnished an extra source of C and N and therefore, partly, complemented the BP in Loch Creran, however, the lability of such DOM was not directly assessed in this study.

Finally, this study demonstrated that a link exists between phytoplankton, the production of DOM and its consumption by heterotrophic bacteria (LeB Williams 2000) but this trophic link is more difficult to identify in coastal areas due to alternative, sometime dominant, sources of DOM, which may explain the absence of direct statistical correlation between the DOM and bacterial variables (BA and BP) in this work.

3.9.2.4 Top-down control of bacterial population and trophic transfer

Another major driver of bacterial activity in aquatic systems is the pressure exerted by microzooplankton grazers (Azam et al. 1983, Sherr & Sherr 1988, Strom

2000). Sherr & Sherr (1991) demonstrated that most of bacterivorous activity occurs among the smallest size classes ($< 10 \mu\text{m}$), with marine HNAN now thought to be the main grazers of bacteria (Strom 2000). The abundance of HNAN has been found to be highly correlated to bacterial abundance in freshwater (Berninger et al. 1991) and marine systems (Sanders et al. 1992). Similarly in this study, HNAN numbers and BA were correlated (Pearson, $p < 0.001$).

Gasol (1994) proposed a qualitative framework to assess the regulation of HNAN populations. He determined a theoretical maximum (TM) and a mean realisable abundance (MRA) of HNAN from the literature that allow the distinction between top-down (below MRA) and a bottom-up (between MRA and TM) controlled HNAN population. Results obtained in this study (Fig. 3.22) suggested that most of the time HNAN abundance was bottom up controlled in Loch Creran (i.e. HNAN abundances depended on bacterial numbers) and consequently that HNAN were regulating BA. Berninger et al. (1991) found similar results with most of their data falling close to, but below the TM.

Although the theoretical model of Gasol gave, in this case, interesting insights on the regulation flow of HNAN abundance and subsequently BA, it makes the critical assumptions that: 1) HNAN feed only on bacteria and 2) that the HNAN response is proportional to BA. First, these assumptions are flawed in three respects: HNAN have been reported to use a variety of sources of energy like picophytoplankton (Sherr & Sherr 1991), viruses (Gonzalez & Suttle 1993), or DOM (Tranvik et al. 1993). Although these food resources might not meet the sole energy requirement of the HNAN population, they might be responsible for the data points found above the TM (e.g. an HNAN abundance greater to what can be supported only by BA). Second, the bacterial population might be subjected to grazing pressure from other marine protists (e.g. small ciliates, choanoflagellates, small nonarmoured dinoflagellates or mixotrophic

dinoflagellates), which would remove part of the bacterial population. For example, small ciliates have been found to graze on bacteria at high rates in productive coastal regions (Sherr & Sherr 1987). Third, the HNAN population might also have experienced predatory control from higher trophic levels. In this study, a strong positive correlation was observed between BA and small dinoflagellates (Pearson, $p < 0.001$) in addition to a negative correlation between HNAN and small dinoflagellates (Pearson, $p < 0.01$). These results suggested that small dinoflagellates had a positive effect on BA by releasing HNAN grazing pressure through predation of HNAN. Similar trends have been observed in several studies carried out in various environments (Sherr & Sherr 1991, Strom 1991, Kuparinen & Bjornsen 1992) and these results illustrate the concept of a “trophic cascade”. Fundamentally, the importance of HNAN grazing on BA resides in the fact that it links the organic matter pool to higher trophic levels by making OM available into a form that would not be otherwise utilisable (Pomeroy 1974, Azam et al. 1983) by larger micro organisms.

3.9.2.5 Phytoplanktonic communities in Scottish coastal waters

The analysis of phytoplankton demonstrated a repeatable annual cycle of phytoplankton species succession (MDS analysis, Fig. 3.28). A succession pattern, related to inorganic nutrient availability typical of temperate waters was found at LY1 and FF, with diatoms dominating the spring bloom followed by the occurrence of ciliates and large dinoflagellates in summer and early autumn. Redundancy analysis confirmed these trends associating species of the spring bloom with nutrient and mixing, whereas summer and early autumn phytoplankters were related to temperature changes. Relative abundance (Fig. 3.25) and correlation with environmental variables (Fig. 3.26 and 3.27) also confirmed the dominance of diatoms in the Firth of Lorne

phytoplankton bloom found in previous studies (Fehling 2004). Phytoplankton composition in Scottish coastal waters reported in this study was similar to observations reported in previous work (Jones 1979, Tett 1992, Fehling 2004). Although, it has been suggested that *Chaetoceros* species, as opposed to *Skeletonema* sp, dominate the phytoplankton spring bloom in Loch Creran (C. Laurent, personal communication), this study identified a possible alternation between *Skeletonema* sp and *Chaetoceros* sp as the dominant species during the spring bloom on the west coast of Scotland. In fact, *Skeletonema* sp was found to dominate the spring bloom mainly at LY1 station, whereas, the dominant species at this time in Loch Creran were *Chaetoceros* sp, *Thalassiosira* sp and *Leptocylindricus* sp (Fig. 3.24).

Annual variability was similar for the four groups of nanoflagellates studied with a short spring increase (March), followed by a late spring (May) and a longer summer bloom. No significant differences were observed between depths and sites. However, all the groups, except other HNAN, exhibited a higher abundance in 2005 than 2004 (section 3.7.2, this Chapter). This smaller proportion of other HNAN in 2005, particularly during the summer, was accompanied by a greater abundance of small dinoflagellates (Fig. 3.19 and 3.20). This observation was confirmed with a negative correlation (Pearson, $p < 0.01$) between HNAN and small dinoflagellates. These observations might have been linked to bacterial grazing and are discussed in section 3.9.2.4 in this Chapter.

The Choanoflagellate, small dinoflagellate, phototrophic and heterotrophic nanoflagellate data collected for this thesis are one the few time series available to date in the west coast of Scotland, exceptions being Navarro et al. (in press) and in temperate coastal waters (Sherr et al. 1986, McManus & Fuhrman 1988, Murrell & Hollibaugh 1998).

In Loch Creran, the highest chl *a* concentrations (8 – 10 mg chl *a* m⁻³) were recorded in April and May 2005, when the phytoplankton community was dominated by *Leptocylindricus minimus*, *Rhizosolenia* sp and small *Chaetoceros* sp. The maximal chl *a* concentration recorded at LY1 occurred in June (~ 4 mg chl *a* m⁻³) when *Leptocylindricus danicus* and small *Chaetoceros* sp were dominating the community of large-celled autotrophs. This therefore corroborated the different dynamics of the phytoplankton community between Loch Creran and the Firth of Lorne mentioned above.

3.10 Conclusion of field study

In conclusion, microbial communities observed on the west coast of Scotland demonstrated seasonality in term of biomass and phytoplankton species succession. Bacteria, within the microbial food web, were shown to be highly dynamic rapidly using autochthonous sources of nutrients (produced by phytoplankton) that were supplemented by external sources through river inputs and sea-loch outflow. These allochthonous sources (river, runoff) of organic nutrients were found significantly greater than potential Fish farm loadings and, although this work did not highlight increased inputs of organic nutrients from the fish farm, it provided insight on the contribution of the farm to DOM stocks in Loch Creran. This study, however, suggested that fish farm did not negatively perturb the pelagic microbial community of Loch Creran. The study of the dynamics of the bacterial population revealed a higher bacterial production in Loch Creran, probably a consequence of more abundant resources available for growth. It also pointed out the importance of accounting for top-down regulation of bacterial population. Thus, using the framework developed by Gasol (1994), the bacterial population was shown to be essentially controlled by grazing.

- CHAPTER 4 -

*Organic C and N stoichiometry and trophic transfer within a
bacteria microflagellate system*

4.1 Introduction

The concept of the Redfield ratio (Redfield 1963) is one of the cornerstones of marine biogeochemistry. Analysis of microbial ecosystems is often based around Redfield dynamics by making the assumption that the atomic ratio of C, N, P is in the proportion of 106:16:1 (Redfield 1963, Brzezinski 1985). It has been postulated that these ratios represent balanced proportions in marine organisms; however, bacterial biomass is N- and P-rich, which results in low C:N and C:P ratios compared to phytoplankton (Bratbak 1985). Any departures from these ratios affect the metabolism of the organisms in terms of growth, nutrient uptake or competition for nutrients (Hecky et al. 1993, Sterner et al. 2002, Kuijper et al. 2004). The growth of phytoplankton (Brzezinski 1985) or bacteria (Fagerbakke et al. 1996), the regeneration of nutrients (Goldman et al. 1985) or the trophic transfer of energy via grazing (Thingstad & Pengerud 1985) are ultimately related to elemental stoichiometry, which, therefore, appears to be critical in microbial food web functioning.

It is still controversial whether or not the stoichiometry of available substrate affects the relative elemental composition of marine bacteria; although fixed specific elemental ratios are still widely utilised in ecological studies or modelling work (Fasham et al. 1990, Anderson & Williams 1998, Anderson & Ducklow 2001). Recent reviews (Doney 1999, Sterner et al. 2002) have further highlighted the need for a better

understanding of this variable nutrient stoichiometry if we are to better understand biogeochemical cycling of elements and microbial food web interactions.

Bacteria compete with phytoplankton for inorganic nutrients but are also capable of utilising organic nutrients in the form of DOM. A significant component of marine DOM is likely to be refractory in nature (Hansell & Carlson 2002), whilst autochthonous DOM produced by phytoplankton (eg. By exudation or lysis of senescent phytoplankton) is likely to be labile and potentially of variable C:N ratio (see Chapter 5 and references therein). Moreover, direct and indirect anthropogenic sources (such as organic matter waste and excess food from fish farm activities) have the potential to modify the concentrations of DOM in seawater and thereby affect organic nutrient availability to microbial communities.

It is often assumed that bacterial C:N stoichiometry is much less variable than that of phytoplankton (Bratbak 1985, Sanders et al. 1992, Fukuda et al. 1998). However, C:N ratios of bacteria in the laboratory have been shown to vary four fold from 3.8 to 15 (Kirchman 2000). In field experiments, bacterial C:N ratios have been found to be less variable, at 3.8 to 9.9 (Fukuda et al. 1998) but still encompass a range that is both smaller and larger than the Redfield value. Hence, variable bacterial C:N has similar potential to that of phytoplankton to influence trophic energy transfer. For example, such variable cell nutrient composition has particular implications for trophic transfer in terms of variability of “food quality” for predators (Hecky et al. 1993, Nagata & Kirchman 1996, Mitra et al. 2003, Kuijper et al. 2004) as well as inorganic nutrient regeneration (Goldman & Dennett 1991, Vadstein et al. 2003). Therefore it appears that the effect of the relative availability of organic C and N on bacterial biomass remains unclear. For example, Goldman et al (1987) found no effect of substrate C:N ratio on bacterial biomass C:N. In contrast, Tezuka (1990) found bacterial C:N ratio to increase with substrate C:N ratio.

Similarly to C:N stoichiometry discussed above, the determination gross growth efficiency (GGE) has been subject to debate (del Giorgio & Cole 1998, Ducklow 2000, Kirchman 2000) as it is affected by the productivity of the studied ecosystem. For example, organisms from oligotrophic regions (low nutrient) tend to exhibit lower GGE than those from more productive systems. This concept, however, has been widely challenged (Anderson & Williams 1998, Geider & La Roche 2002) and it remains unclear whether or not accurate measurements of GGE, and therefore an accurate understanding of C cycling in the Ocean, are achievable.

An important pathway of energy transfer within pelagic communities is the grazing on bacterial populations by protists (Sherr & Sherr 1988). Nano-zooplankton are the major grazers of bacteria, providing a crucial trophic link by ingesting an important part of the bacterial production (Lancelot & Billen 1984, Sherr & Sherr 1991, Strom 2000). However, the influence of a variable elemental composition of bacteria on trophic transfer to nanoflagellate grazers, and hence the efficiency of this trophic link in terms of C and N flux, remains poorly understood. In fact, nutritional quality has been highlighted as an important factor in determining the rate of ingestion of prey by protistan predators (Flynn et al. 1996) and also the rate at which nutrients are regenerated (Goldman et al. 1985, Goldman et al. 1987, Goldman & Dennett 1991). The work of Thingstad and Pengerud (1985) and Thingstad (1987) indicates that, should bacterial stoichiometry be variable, then nutrient cycling in predator/prey scenarios will be modified, leading, for example, to different nutrient regeneration scenarios (Goldman et al. 1987). Microflagellates grazers, such as *Paraphysomonas* sp, are thought to maintain a constant C:N stoichiometry and therefore, if bacterial stoichiometry is unbalanced, grazers retain the nutrient in the shortest supply.

This chapter presents a laboratory experiment to determine the influence of organic substrate C:N ratio on bacterial cellular C:N, and the subsequent trophic transfer

of this organic material to nano-flagellate grazers. The experiment was designed to test two hypotheses, (1) that variable organic C:N ratios affect the bacterial biomass stoichiometry and the bacterial growth efficiency, and (2) that variable organic C:N ratios affect the subsequent transfer of nutrients to nano-zooplankters through grazing. To test these two hypotheses, the marine bacterium, *Vibrio natriegens*, was grown in the laboratory under a variety of organic C and N concentrations. This bacterial species was also grown in the same nutrient conditions but in the presence of a nano-flagellate grazer, *Paraphysomonas vestita*, to investigate the transfer of C and N through a simple trophic link.

4.2 Cultures and Experimental design

4.2.1 Experimental design

The first step of the experiment was the incubation of the bacterium *Vibrio natriegens* in a gradient of organic nutrient concentration. Subsequently, the same bacteria was incubated in identical media, but in the presence of a predator *P. vestita* (Stokes) De Saedeleer. *V. natriegens* was chosen because it is a well known marine bacteria that has been previously used in study of bacterial metabolism, growth and biomass stoichiometry (Bratbak 1985, Goldman et al. 1985, Goldman et al. 1987), and in grazing experiments (Nagata & Kirchman 1992). Vibrionaceae are relatively large, rod-shaped, bacteria (0.8-1 μm) and are easily recognisable under a microscope after DNA staining. *Paraphysomonas* sp. has also been the organism of choice in previous laboratory grazing studies, ingesting either phytoplankton (John & Davidson 2001, Davidson et al. 2005) or bacterial (Nagata & Kirchman 1992) prey. Nutrient regeneration studies and investigations of nano-flagellate stoichiometry have also utilised the *Paraphysomonas* genera (Sin et al. 1998, Mitra et al. 2003). *P. vestita* is a

spherical cell (7-10 μm diameter) therefore easy to identify and count. *P. vestita* also grows quickly in cultures with artificial media (John & Davidson 2001)

For each experiment, six duplicated (12 flasks in total) two-litres Erlenmeyer flasks were filled with 1 L of a sterile (autoclaved 15 min at 120°C), artificial standard seawater media “ESAW” (Harrison et al. 1980) and of these 12 flasks, ten received organic media (Table 4.01). The two remaining flasks were filled only with ESAW and were used as control. ESAW was used to avoid any detrimental organic nutrient background. Bacteria alone, and bacteria and predator, were added in each flask at a concentration of 1×10^6 cell ml^{-1} for *V. natriegens* and 1×10^4 cell ml^{-1} for *P. vestita*. The volumes of inoculums were determined the day before the experiment started based on cell counts from stock cultures. All experiments were conducted in duplicate. Simple organic compounds, arginine and glucose, were used to achieve a gradient of bioavailable organic substrate C to N ratio (C:N_S) that created potentially different limiting conditions (Table 4.01). Organic nitrogen concentration was kept constant, with the concentration of glucose varying to achieve the C:N_S ratio desired (1.5:1, 3:1, 6:1, 9:1 and 15:1). Low C:N_S were carbon-deficient and therefore C-limiting conditions whereas high C:N_S lacked nitrogen and generated N-limiting conditions. In between these two conditions, a Redfield C:N_S of 6:1 was, *a priori*, used to represent balanced proportions between organic carbon and nitrogen.

4.2.2 Cultures of prey and predator

Vibrio natriegens (MMB) were concentrated from axenic stock culture (250 ml) in 50 ml sterile polypropylene centrifugation tubes and centrifuged at 4500 rpm for 8 minutes. The supernatant was removed from each tube with a sterile 10 ml pipette, in 5 ml aliquots, until 5 to 10 ml remained with the bacterial pellet. The first 5 ml were

gently aspirated by pipette to break up the pellets, which were subsequently resuspended in 50 ml of sterile ESAW. The operation was repeated three times to obtain a dilution of 99.9 % of the initial bacterial media and prevent carry over of residual organic nutrients from the stock cultures.

Paraphysomonas vestita inoculums (CCAP 935/14) were prepared according to Davidson et al. (2005). Fifty ml sterile polypropylene centrifugation tubes were filled with *P. vestita* stock cultures and centrifuged at 1500 rpm for 10 minutes. The supernatant was carefully removed using 10 ml sterile pipette to leave a volume of five ml with the pellet. The pellet was aspirated as described above for bacteria and cells were resuspended in 50 ml sterile ESAW.

Experiments were carried out in the dark, at 15°C for a duration of 13 days. Both bacteria alone and bacteria + nano-flagellates flasks were sampled and analysed in identical manner. Inoculums of both *V. natriegens* and *P. vestita* were prepared less than 24 hours before the start of the experiment and kept at experimental conditions.

4.2.3 Cell counts and chemical analysis

The abundance of *P. vestita* was determined by light microscopy using a Sedgewick Rafter cell (1ml). Replicated sub-samples for cell counts were taken everyday in sterile conditions under a laminar flow cabinet. Sub-sample (5ml) was fixed with lugol iodine (final concentration 1%) and stored in the dark at 5-6°C. Samples were counted on the day of collection to allow of cell growth to be monitored. At least 10 squares (or 200 cells minimum) were counted.

Table 4.01. Composition of the experimental medium with details of C and N concentrations.

C:N _s	Control	1.5:1	3:1	6:1	9:1	15:1
arginine* ml	0	0.25	0.25	0.25	0.25	0.25
glucose* ml	0	0	0.25	0.75	1.25	1.5
N (μM)	0	100	100	100	100	100
C (μM)	0	150	300	600	900	1500
C(μM) arginine	0	150	150	150	150	150
C(μM) glucose	0	0	150	450	750	1350
%C from Glucose	0	0	50	75	83	90
ESAW [‡] ml	250	250	250	250	250	250
ELGA water ml	750	750	750	750	750	750

* Stock solution of glucose & arginine at 100 mM, i.e. add 1mL/L to reach a final concentration of 100 μM

‡ Stock ESAW at 200 % i.e dissolved at ¼ with RO water to obtain normal ESAW.

Vibrio natriegens was determined by epifluorescence microscopy as described in Chapter 2, section 2.4.1.4. Duplicated sub-samples (5ml) were taken each day in sterile conditions under a laminar flow cabinet and fixed with glutaraldehyde (final concentration 4%). Samples were stored in the dark at 5-6°C until DAPI staining.

Particulate organic Carbon (POC) and Nitrogen (PON), Dissolved organic carbon (DOC) and nitrogen (DON) and dissolved inorganic nitrogen (ammonium, nitrate and nitrite) were measured every two or three days. POC and PON samples (one per flask and per sampling occasion) (30ml) were analysed as described in section 2.3.2.2 of Chapter 2. The filtrates (30ml, from POC and PON samples) were used for inorganic nutrient analysis (see section 2.3.1 in Chapter 2). DOC and DON were analysed by HTCO (see section 2.3.2.1 in Chapter 2). The filters (GF/F or A/E) used in this study were pre-combusted (650°C for 6 hours) to reduce the nominal porosity to ~ 0.4 μm . Typical *Vibrio* cells have a diameter of about 0.8 to 1.0 μm , and around 3-8 μm for *P. vestita*, therefore most of the bacteria and microflagellates were retained onto the filters (Nayar & Chou 2003).

4.3 Results

4.3.1 *Vibrio natriegens* Carbon and Nitrogen dynamics

In the initial experiment, bacteria were grown in the absence of the predator with a range of organic substrate C:N ratios. All substrate C:N ratios (C:N_S) supported bacterial growth. This bacterial growth was reflected in the decrease of DOC and DON (due to bacterial uptake) and the increase/assimilation in POC and PON (due to bacterial assimilation, see Fig. 4.01) with time. POC and PON attained a maximum between day six and 13, with a similar peak in NH_4^+ due to nutrient regeneration.

DOC and DON concentration decreased rapidly until day nine then remained low towards the end of the experiment. Only three of the six treatments were measured for DOC and DON concentrations due to instrument failure. For the highest C:N_S (15:1), DOC concentrations declined to around 8 mg C L⁻¹, whereas the two other treatments shown (1.5 and 6) had a lower final concentration around 2 mg C L⁻¹. The amount of DOC taken up increased with C:N_S from 2.9 to 7.4 mg C L⁻¹, whereas, for DON, it remained in the same range (0.9 – 1.7 mg N L⁻¹) independent of substrate stoichiometry (Table 4.02).

POC variations with time ranged from 0.21 mg C L⁻¹ to 2.07 mg C L⁻¹, the smallest increase recorded being for the lowest C:N_S (Fig. 4.01-c). Flasks amended with C:N_S of 6, 9 and 15 exhibited a similar pattern, reached maximum values of POC at the same time and corresponded to similar maximal POC concentrations at day 6 (~ 2 mg C L⁻¹). PON reached a maximum (0.15-0.25 mg N L⁻¹) on day nine for all C:N_S except for C:N_S of 3:1 that reached a maximum on day 6. The highest PON values were observed for C:N_S of 6 and 9 (Fig. 4.01-d).

The average value of bacterial C:N at inoculum was 4.49 ± 0.63 . In all cultures, bacterial C:N increased with time (Fig. 4.01-f) to reached a maximum around day four, then decreased toward the end of incubations. Bacterial C:N (C:N_P) also increased with increasing C:N_S (Fig. 4.01-f). Furthermore, C:N_P values were similar (at 4-6) for low C:N_S (1.5 and 3) or high (~10-12) C:N_S (9 and 15) with mid values (of ~ 8) displayed for a C:N_S close to Redfield ratio (~6). Bacterial cell density (not shown) peaked at day 4 for all C:N_S with a maximum of 1.5×10^7 cell L⁻¹ (C:N_S = 15:1).

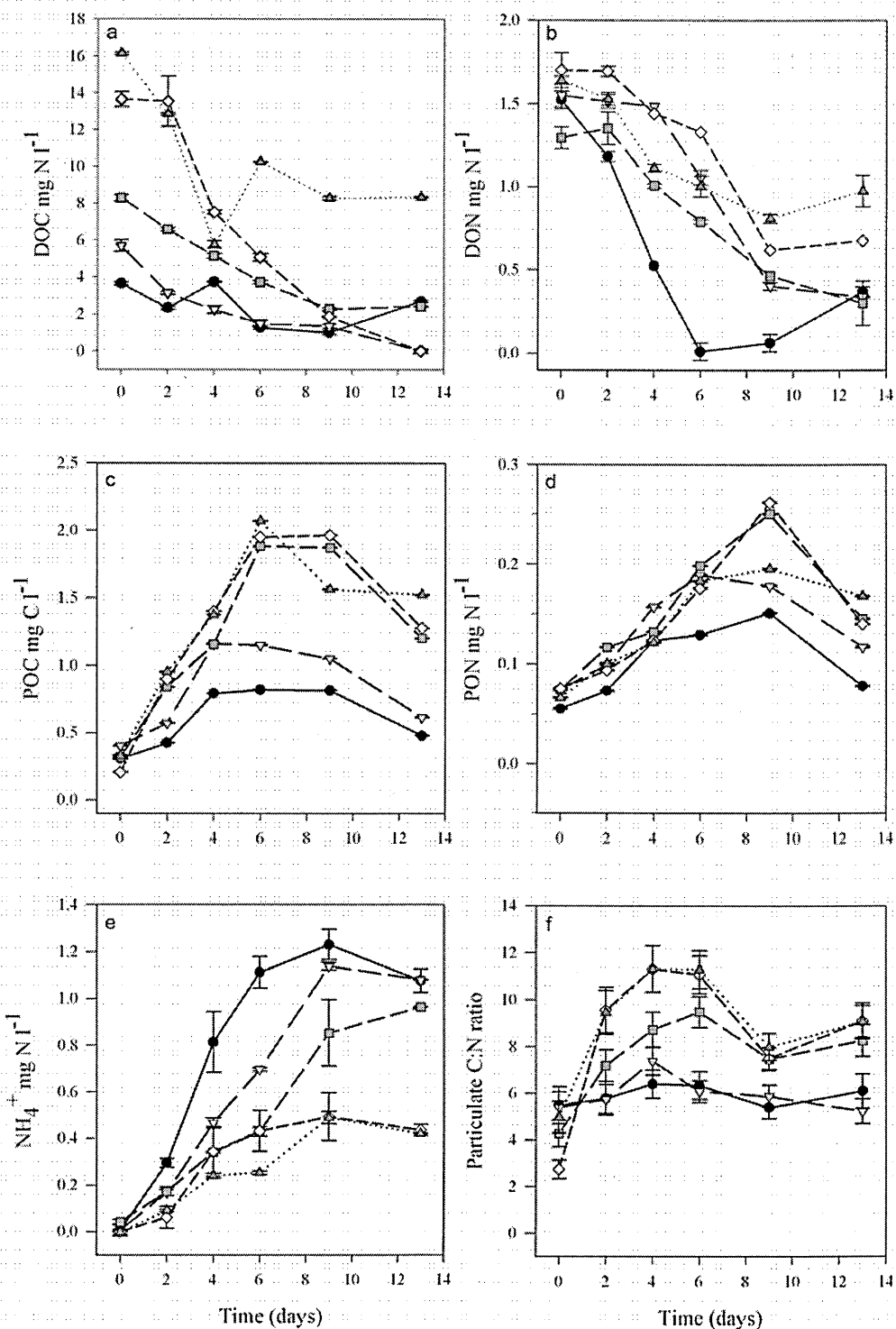


Figure 4.01. DOC (a), DON (b), POC (c), PON (d), particulate C:N (e) and NH₄⁺ (f) for *Vibrio natriegens* incubations grown under different substrate C:N ratio conditions. Symbols represent the different C:N_s ratios with 1.5:1 (black circle), 3:1 (triangle down), 6:1 (square), 9:1 (diamond) and 15:1 (triangle up). Error bars are ± S.E. from duplicates

4.3.2 *V. natriegens* and *P. vestita*: C and N dynamics when heterotrophic predator present

In the second experiment, *V. natriegens* were incubated in presence of a predator, *P. vestita*, with the same range of organic substrate C:N ratios as used in the first experiment. In contrast to the first experiment, DOC concentrations decreased to a minimum of approximately 2 mg C L⁻¹ at the same time (day four) for the three treatments shown (1.5, 6, 15) and remained at this low concentration until the end of the experiment (Fig 4.02-a).

The quantity of DOC taken up (Table 4.02) exhibited the same pattern as for *V. natriegens* alone, in that it increased with increasing C:N_S. However, these quantities covered a greater range of values (1.9 – 13.5 mg C L⁻¹).

DON decreased during the same period (Fig. 4.02-b), with a minimum value of 0.0 – 0.2 mg-atoms N L⁻¹ reached at day six and with no major differences recognised between treatments. This response was concomitant to the uptake of DON (Table 4.02), of which values remained between 1.2 – 1.4 mg N L⁻¹ (similar to *V. natriegens* alone, 0.9 – 1.5 mg-atoms N L⁻¹). DON uptake was independent of C:N_S. As for bacteria alone, POC attained maximal values (0.97 – 4.99 mg C L⁻¹) at day four (~cell peak) for all C:N_S. Similar to *V. natriegens* alone, media with low C:N_S (1.5 and 3) exhibited the smallest POC increase (Fig. 4.02-c), whereas, highest POC values were associated to high C:N_S (9 and 15). However, C:N_S of six fell in the middle of these POC values, which is different from the response with *V. natriegens* alone where flasks with C:N_S of six, nine and 15 were close. A very similar pattern was recognised for PON (Fig. 4.02-d) with lowest values in flasks with C:N_S of 1.5 and 3, mid concentration for C:N_S of 6 and highest N biomass recorded for C:N_S of 9 and 15.

Table 4.02. Mass balance of Carbon and Nitrogen , growth gross efficiency and N regeneration rate in cultures grown on various combinations of carbon and nitrogen ratios.

	C:N substrate	Uptake DOC ^a	Δ-POC	GGE carbon	Uptake DON	E _N [*]	N efficiency	Part. C:N
		mg C L ⁻¹	mg C L ⁻¹	%	mg N L ⁻¹	μg N [mg C] ⁻¹ h ⁻¹	%	
<i>V. natriegens</i>	1.5:1	2.9	0.5	19.2	1.5	16.9	84.8	5.4
	3:1	4.3	0.7	21.6	1.7	9.2	54.7	5.9
	6:1	6.5	1.6	32.4	0.9	2.4	55.2	7.5
	9:1	11.8	1.7	20.3	1.6	2.6	23.6	7.5
	15:1	7.4	1.7	32.1	0.9	0.7	30.9	8.0
<i>V. natriegens</i>	1.5:1	1.9	0.5	27.2	1.4	16.6	57.6	5.3
	3:1	3.0	0.7	22.4	1.4	8.9	55.0	5.8
	6:1	6.9	1.7	24.1	1.2	3.2	45.5	6.5
<i>P. vestita</i>	9:1	9.6	3.2	33.8	1.2	1.0	46.3	8.0
	15:1	13.5	3.6	26.4	1.4	0.6	40.3	8.6

^a DOC uptake calculated as the difference in concentration between day zero and day of maximum biomass (POC) observed.
^{*} Nitrogen regeneration efficiency calculated according to Goldmann et al (1987) between Day 0 and Day 6.

The C:N stoichiometry of the particulate pool (Fig. 4.02-f) varied with time with an initial increase to day four to six, then decreasing till day nine but not for C:NS of 1.5 that carried on increasing till the end of the experiment. C:N_P ranged between 4.8 and 8.6 with the highest values observed for the highest C:N_S (9 and 15).

Bacterial abundance was greatest (7.8×10^6 cell L⁻¹, C:N_S = 15) at day five. However, bacterial abundance was on average approximately half of that obtained when *V. natrigens* was alone. *P. vestita* abundance increased until day seven to nine, reaching 3.3×10^6 cell L⁻¹ for the highest C:N_S.

4.3.3 Maximum biomass, C:N_P and NH₄⁺

In the prey only experiment (first experiment), the concentration of regenerated ammonium increased with time (Fig. 4.1-e), however the magnitude of this regeneration decreased with increasing C:N_S. A similar pattern was observed in the prey and predator experiment (second experiment), where NH₄⁺ concentrations peaked at day six for all treatments (Fig. 4.02-e) and remained approximately constant till the end of experiment ($0.6 - 1.2$ mg-atoms N L⁻¹). The inverse correlation of NH₄⁺ regeneration and increasing C:N_S was corroborated by the relation observed in Figure 4.03. This figure depicts the changes in maximum yield of POC, PON, NH₄⁺ and maximum C:N_P with changing C:N_S.

Maximum concentration of NH₄⁺ decreased with increasing C:N_S (Fig. 4.03-a and b) for both experiments (*V. natrigens* alone and *V. natrigens* + *P. vestita*). This trend was inversely correlated with PON concentration that increased with increasing C:N_S. POC, at time of maximum biomass, also increased with C:N_S. C:N_P (of bacteria alone or bacteria + grazers) increased with C:N_S even if C:N_P were generally greater at cell peak than at the end of the experiment. However, the range of C:N (5 to 12)

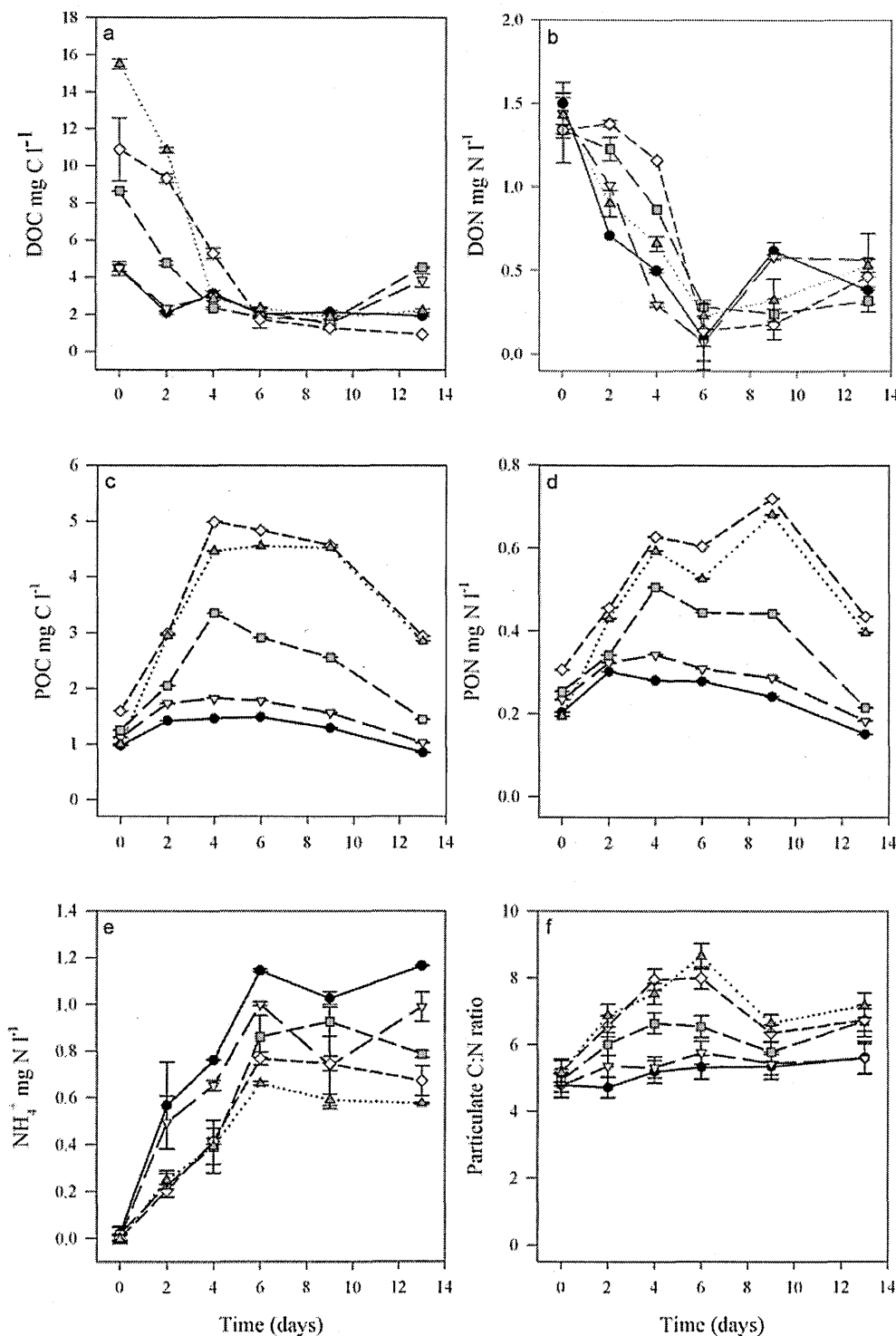


Figure 4.02. DOC (a), DON (b), POC (c), PON (d) particulate C:N (e) and NH₄⁺ (f) for *Vibrio natriegens* and *Paraphysomonas vestita* incubations. Symbols represent the different C:N ratios with 1.5:1 (black circle), 3:1 (triangle down), 6:1 (square), 9:1 (diamond) and 15:1 (triangle up). Error bars are SE from duplicates

displayed by bacteria alone (Fig. 4.03-a) were higher than those (5 to 8) found with bacteria associated with the predator (Fig. 4.03-b).

4.3.4 Carbon Gross Growth Efficiency and Nitrogen regeneration

Gross growth efficiency (GGE) was calculated as the amount of biomass (POC) produced divided by the amount of DOC taken up over the same period of time (day zero to six when bacteria were alone and day zero to 4 when the predator was also present). GGE was low for both systems studied, with on average 25.1 % for *V. natrigens* alone and 26.8 % for *V. natrigens* + *P. vestita* (Table 4.03).

However, no variations of GGE with C:N_S was observed and therefore GGE was not significantly influenced by C:N_S.

Biomass specific regeneration rate of nitrogen (in the form of NH₄⁺) was estimated according to the C:N mass balanced model of Goldman et al. (1987) who proposed that N regeneration is related to substrate, C:N_S, and particulate, C:N_P, ratio:

$$E_N = C_T \left(\frac{1}{C:N_S} - \frac{GGE}{C:N_P} \right), \quad \text{equation 4.01}$$

along with C_T, the rate of carbon uptake (calculated as DOC concentration decrease per unit of time) and GGE. E_N ranged between 1 and 17 μg N (mg C)⁻¹ h⁻¹ (Fig. 4.04-a). The major feature of N regeneration rate (Fig. 4.04-a) was its decrease with increasing C:N_S. More surprisingly, this N regeneration rate was similar in both experiments, when one would expect the predator-prey system to be more efficient in terms of N regeneration.

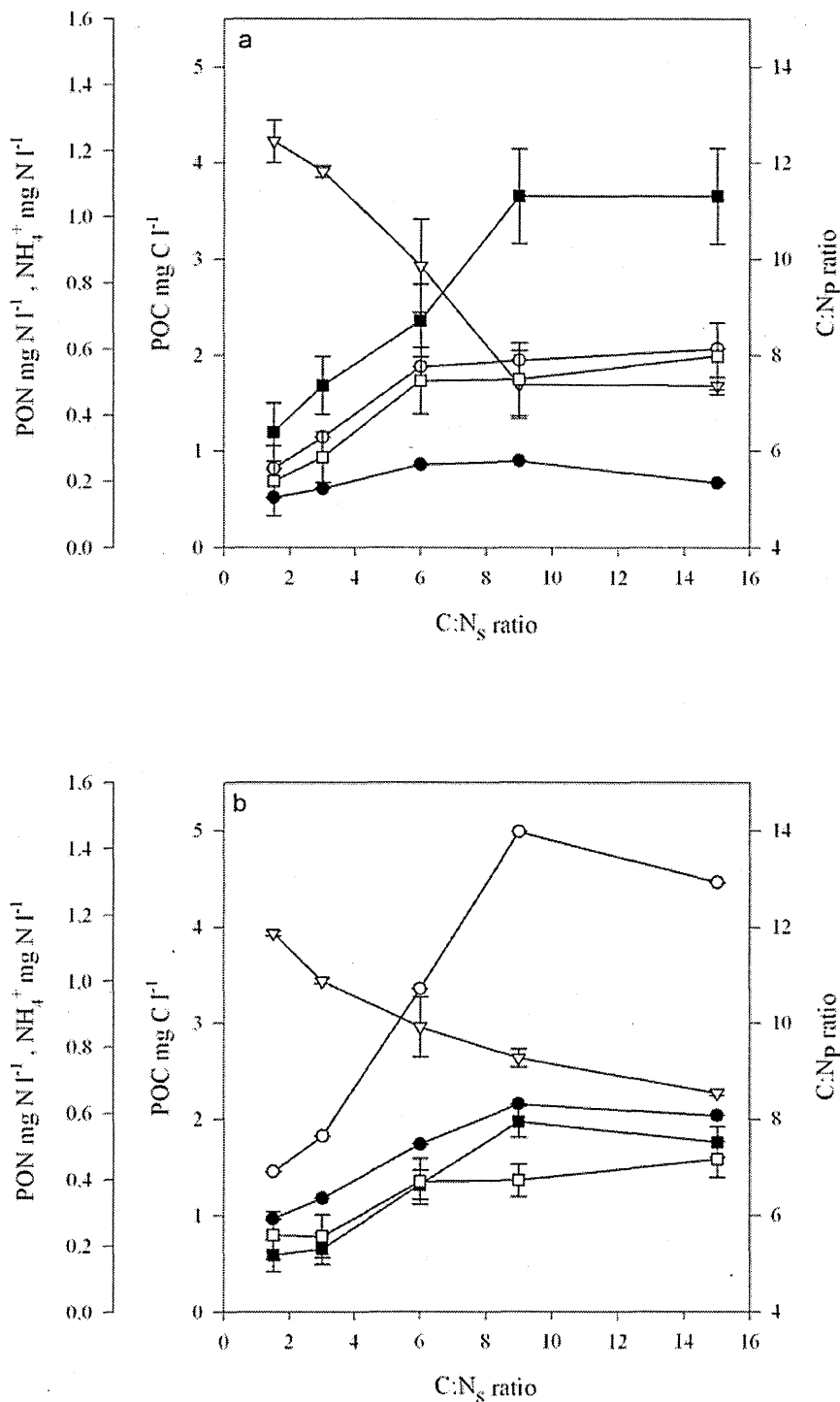


Figure 4.03. Maximum values of NH_4^+ (∇), POC (\bullet) and PON (\circ) concentrations, along with C:N_p at cell peak (\blacksquare) and C:N_p at the end of the experiment (\square) in *Vibrio natriegens* (a) and *V. natriegens* + *Paraphysomonas vestita* (b) inoculums grown under different substrate C:N ratio conditions. Error bars are SE from duplicates

Δ -POC (Fig. 4.03-b) was calculated as the difference between POC at T_{zero} and POC at maximum biomass (day 4 for *V. natriegens* alone, day 5 for *V. natriegens* + *P. vestita*) and plotted for each C:N_S. Generally, Δ -POC increased sigmoidally with increasing C:N_S. Below a balanced Redfield value of C:N_S(6:1), the variation of POC was similar in both *V. natriegens* alone and *V. natriegens* + *P. vestita*. However, above a C:N_S of 6:1, Δ -POC diverged in the two treatments, reaching value of 1.5 mg C L⁻¹ when *V. natriegens* was grown alone whereas Δ -POC was higher (3.5 – 4.0 mg C L⁻¹) when the predator was present.

Particulate C:N ratio at time of maximum biomass was always greater than five and increased with increasing C:N_S (Fig. 4.04-c). The slope of the linear regression was higher when bacteria were grown with predator than when bacteria grown alone (Regression line are: C:N_P = 0.188 x C:N_S + 5.56, $r^2=0.71$, $p<0.01$ and C:N_P = 0.255 x C:N_S + 5.08, $r^2=0.93$, $p<0.01$, for *V. natriegens* and *V. natriegens* + *P. vestita* respectively).

4.4 Discussion

4.4.1 Variability of C:N ratio in prey predator system

The first hypotheses investigated in this experiment was the effect of organic substrate carbon to nitrogen (C:N_S) ratios on bacterial carbon to nitrogen stoichiometry (C:N_P). In aquatic systems, C:N stoichiometry may govern whether a system will be C limited (low C:N_S) or N limited (high C:N_S) (Redfield 1963). In this experiment, C:N_S of 6:1, 9:1 and 15:1 generated similar bacterial biomass (POC) of ~ 2 mg C.L⁻¹. These C:N_S exceeded the bacterial C:N_P of 4.5:1 proposed by Bratback (1985) and Fagerbakke

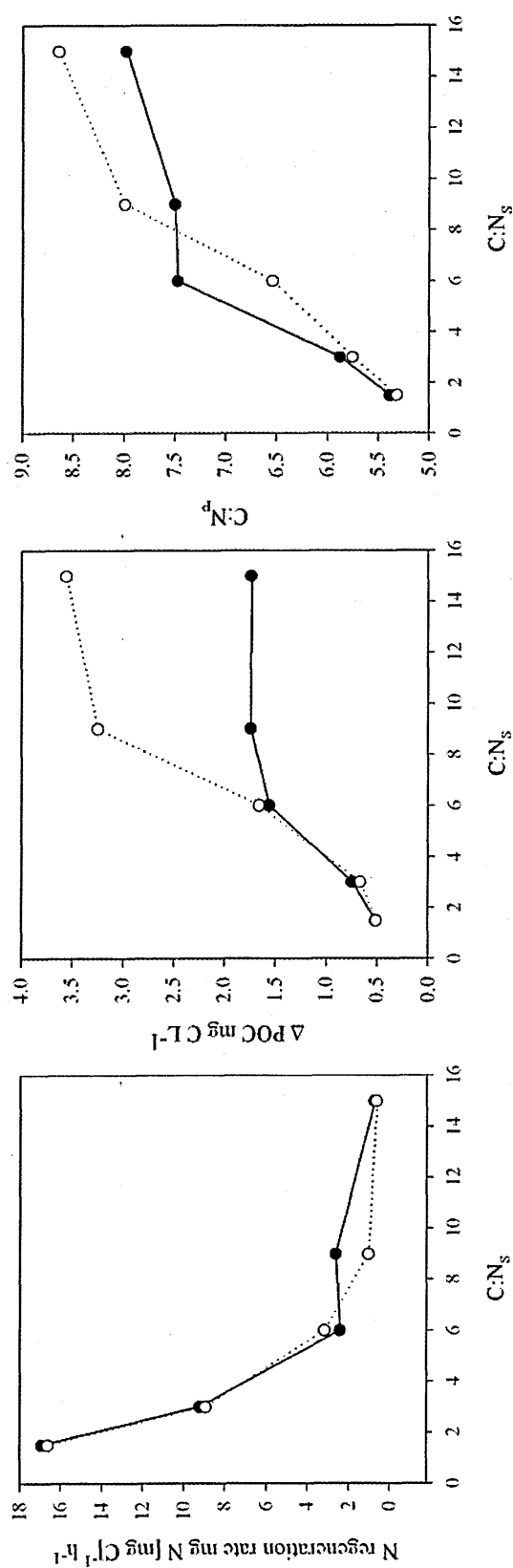


Figure 4.04. Biomass specific N regeneration rates (a), ΔPOC (b) and C:N_p (c) for each C:N_s in *Vibrio natriegens* (●) and *V. natriegens* + *Paraphysomonas vestita* (○) experiments.

(1996) as a metabolically stable cellular C:N for bacteria. Therefore, the supposedly balanced Redfield value of 6:1 produced a N-limited condition for bacteria hence the similarity in response at this C:N_S (6:1) with C:N_S of 9:1 and 15:1 (also N-limited conditions). The results are consistent with Goldman (1980) who previously suggested that Redfield ratio of 106:16:1 does not offer nutritionally balanced conditions for bacteria (as compared to phytoplankton or other marine organisms).

The variation of cellular elemental composition (C and N) with varying nutrient ratios has been long debated and is still a matter of controversy (Bratbak 1985, Goldman et al. 1985, Goldman & Dennett 2000, Kirchman 2000). Furthermore, studies using organic C and N as substrate are rare (Tezuka 1990) and studies investigating the subsequent trophic transfer of produced biomass are required. Goldman et al. (1985) argued for an invariant bacterial C:N, regardless of substrate C:N. They found a low and invariant C:N of bacteria (5.1 ± 0.57) even with a 10-fold change in substrate C:N. Later, however, Goldman et al. (2000), revised their statements in favour of variable bacterial C:N, reporting values ranging from 4.5 to 7 in batch cultures and 4.5 – 11 in continuous cultures. Tezuka (1990), in a study that utilised both organic C and N, demonstrated that bacterial C:N increases with increasing substrate C:N. In accordance with Tezuka (1990), this work demonstrated bacterial C:N ratio to increase with increasing C:N_S (i.e. with increasing N limitation). This was confirmed by a significant linear relationship ($r^2=0.78$ and p value < 0.05) found between bacterial C:N and substrate C:N. C:N_P values observed (from 5.3 to 8.6) were in the range of value reported in previous studies (Kirchman et al. 1982a, Fukuda et al. 1998) and, hence, further question the assumed constancy of bacterial C:N.

When the grazer was present, a similar linear relationship was found between C:N_P and C:N_S ($r^2=0.94$ and p -value < 0.01), which was close to that obtained when

Table 4.03. Summary of bacterial C:N ratios, N regeneration and gross growth efficiency.

Substrate	Organisms	CN _B	N regen. %	GGE %	References
Mixture of glucose-aa-inorganic N	bacteria	4.2 – 8.7	0.1 to 70	23.9 – 89.3	Goldmann et al. (1987)
Mixture of glucose-aa-inorganic N	Natural assemblage of marine bacteria	4.1–6.4	3–87 (31–74)*	36–65	Goldmann et al. (1991)
Mixture of Glucose and Arginine	<i>Vibrio natriegens</i> and <i>Paraphysomonas vestita</i>	5.3 – 8.6	30.9 – 84.8	19.2 – 33.8	This study
Bacteria with different C:N	Isolate of flagellate form Lake Biwa	2.6 – 5.3	-	-	Nakano (1994)
Glucose, asparagine and glycerol phosphate	Natural assemblage of freshwater bacteria	4.4 – 17.2	0–78	-	Tezuka (1990)
Phytoplankton prey of variable C:N	Protozoan <i>O. marina</i>	-	23–75	-	Davidson et al. (2003)
	Natural assemblages of marine bacteria	Oceanic: 6.8 ± 1.2 Coastal: 5.9 ± 1.1	-	-	Fukuda et al (1998)

* N regeneration calculated from Goldmann et al. (1987)

V. natrigens were grown alone (Fig. 4.04-c). This indicates that the C:N of the combined bacteria and grazer system increased with C:N_S and that the predator did not modulate the C:N of the system to balance proportions but exhibited the stoichiometry of the food it received.

4.4.2 C:N stoichiometry and GGE

The GGE of the bacteria and microflagellate system may be governed either by the C:N stoichiometry of the substrate, or by the limiting nutrient defined as that in the least relative nutrient supply in term of Redfield stoichiometry. Kroer (1993) reported that bacterial GGE decreases with increasing C:N_S in continuous cultures, however this decrease in GGE occurred over a very small range of C:N_S (6.6 – 7.4). Similarly, Goldman and Dennett (2000) found GGE of natural bacteria to be high when a single N source was used and they found that GGE was also high when C:N_S was low (<3:1). They concluded that GGE of 50% should be expected when both N and C sources are readily available to bacteria. However, this bacterial GGE (calculated when bacteria were alone) was independent (linear regression: $GGE = 0.5 \times C:N_S + 22.5$, $r^2 = 0.22$, $p\text{-value} > 0.05$) of C:N_S even at very low C:N_S (1.5:1). Calculated GGE (19 to 32 %) fell in the lower range of commonly accepted values for this parameter (see Table 4.03) despite readily available sources of C (glucose) and N (arginine).

GGE did not vary with substrate stoichiometry, it may vary with substrate complexity or availability (Benner et al. 1992, Goldman & Dennett 2000). Increasing substrate complexity may force bacterial cells to allocate energy differently to overcome costs of enzyme production or active nutrient transport. This might have been the case in our experiment as simple C and N sources were utilised hence bacterial cells directed energy to growth. Furthermore, the use of arginine as sole source of nitrogen in this

experiment could explain the low GGE observed. This would be consistent with the results of Hollibaugh (1978) who found the uptake of arginine (C:N = 1.5:1) to lead to the lowest GGE (~30-32 %) of all substrates studied.

Along with C:N_S and the form of available C and N, C:N_B appears as a major determinant of GGE (del Giorgio & Cole 1998). Goldman et al. (1987) stipulated, based on their model (equation 4.1), that C:N_S governs GGE and therefore N regeneration efficiency. In the experiment with bacteria alone, C:N_P is not constant and encompass large variation (4 – 11). Hence, although Goldman's model simulated our data (similar trend to Goldman's data for E_N v.s. C:N_S), this was because of increasing C:N_P over exponential growth duration (day 0 to day 6-9), and not because of decreasing GGE as suggested by those authors.

It is not impossible to think that GGE could be species specific therefore, as only *V. natriegens* was used in this study, a constant GGE was observed and the regulation mechanism (in the model of Goldman) would be led by C:N_P not GGE. The effects on GGE of phylogenetic composition have already been suggested but are very poorly understood (del Giorgio & Cole 1998, del Giorgio & Cole 2000).

The findings described in this Chapter are not, however, in disagreement with the concept of carbon conservation where C taken up is retained by the cell in proportion to C limitation and that only a stoichiometrically fixed quantity of N is utilised under such conditions. In fact, the rather constant and low GGE found in this experiment and the similarity in the C:N stoichiometry of bacteria or bacteria and grazer may have been hidden by fast regulation at cellular level as exhibited by the important variations (between 5 and 12) of particulate C:N with time (Fig. 4.01-f and 4.02-f).

4.4.3 Coupling C:N_S and N regeneration

Results from this experiment confirmed the relationship between C:N_S and E_N, as regeneration rates increase with decreasing C:N_S. This relationship is well described by the equation 4.1 (at least for exponential growth), and is in agreement with other studies (Hollibaugh 1978, Van Wambeke & Bianchi 1985, Goldman et al. 1987). One of the major findings of Goldman et al. (1987) was a threshold of C:N_S 10:1 under which regeneration of N by growing bacteria occurs. In this study, however, E_N was low but still finite for C:N_S of 15:1. In his work, Goldman stipulated that the C:N ratio of the carbohydrate source of C and the amino acid and protein source of N will determine N regeneration of bacteria. However organic N in nature cannot meet the N requirements of bacterial growth that have to be supplemented by inorganic N (Kirchman et al. 1982b, Wheeler & Kirchman 1986). Hence, it may be possible that, when C:N_S is greater than 10 (e.g N poor), N excretion (in the form of ammonia) should not occur. However, this N excretion occurred in this experiment with C:N_S greater than 10 (i.e. C:N_S = 15:1), which suggests that the C:N ratio of 10 cannot be use as a threshold for N regeneration. Kirchman (2000) also identified similar strong coupling between C:N and N regeneration, along with C:N_S and C:N_B and defined “breakeven points”, where net excretion or uptake of ammonia occur, however he concluded that this C:N mass balance model cannot explain N fluxes in natural systems.

4.4.4 Grazers as a regulatory feedback mechanism

N regeneration was similar with or without grazers reaching values of 1.2 mg L⁻¹ for the lowest C:N_S. Nitrogen recovery (defined as the amount of N assimilated + regenerated divided by the amount of DON taken up), however, was enhanced when

grazers were present. Davidson et al. (2005) described an increasing E_N with decreasing C:N ratio of substrate (preys in his study). This was also found in this experiment with the increasing trend of particulate C:N with increasing $C:N_S$.

In these experiments, the GGE of the system was found to be poorly influenced by substrate C:N. The similarity in the average of GGE, whether or not grazers were present (24 vs 25 %, without and with grazer respectively), confirmed the fact that the growth of the system is ultimately governed by the “primary” resource of the system.

The variation of POC with increasing $C:N_S$ (Fig. 4.04-c) did not differ between the first experiment (prey alone) and the second experiment (prey-predator system). This suggested that, under 6:1, the nutritional conditions did not support sufficient bacterial growth and the subsequent growth of the flagellate. However, $C:N_S$ of 6:1 and above generated greater biomass in the prey predator system (Fig. 4.04-b). Similar patterns were found by Goldman et al. (1991) where Δ -POC increased with increasing $C:N_S$ according to a linear relationship. Differences in $C:N_P$ in the presence and absence of grazers (Fig. 4.01 and 4.02, f) demonstrated the key importance of the flagellate in regulating the stoichiometry of the prey-predator system by preventing excessive increase of $C:N_P$ (maximum value of $C:N_P$ of 12 and 8 without and with predator respectively).

The uptake of DOC was greater when grazers were present (Fig. 4.01 and 4.02, a) as DOC was exhausted at day 5 for all $C:N_S$. In this experiment, it seems likely that the difference in C uptake was due to a faster turn over of bacterial population as bacterial biomass was cropped by heterotrophic grazers, hence, leading to further consumption of DOC by bacteria, compared to when bacteria were grown alone. It has also been suggested that marine microflagellates are able to uptake directly organic compounds of the size of polysaccharides or proteins (Tranvik et al. 1993) which could account for some of this greater consumption of organic substrates.

4.4.5 Effect of potential phosphorus limitation, implications for growth and N regeneration

Thingstad and Pengerud (1985) stated that bacterial growth is mainly driven by grazing and inorganic nutrients, such as phosphorus, not just by organic C resources. In this experiment, flasks were not amended with inorganic phosphorus and, therefore, the potential for phosphorus limitation cannot be neglected. However, the presence of P was revealed by subsequent measurements. Vrede et al. (2002) found P limitation to give the largest cells (5 to 10 times bigger than C limitation) with the highest C content (2 to 3 times) and the highest average C:N (9.5 ± 1 v.s 3.8 for C limited and 7.5 for N limited). The C:N_P ratios observed here were in the range of values reported in other studies carried out in P replete conditions (Bratbak 1985, Goldman et al. 1987). Furthermore, C and N content (from POC and PON values) were closer to what has been found in non P-limited cultures (Nakano 1994, Fukuda et al. 1998). In addition, if the system was phosphorus limited, the uptake of N (and C) would have stopped with P exhaustion. Therefore it is unlikely that bacteria and grazers experienced P limitation in this experiment.

4.5 Conclusion

This set of experiments emphasize the importance of organic substrate C:N ratio in microbial system. The C:N_S and C:N_P were closely linked for both bacteria alone and bacteria and grazer and the increase of C:N_P with increasing C:N_S demonstrated the influence of substrate upon the stoichiometry of organisms. No effects of C:N_S on GGE were found and the variation of particulate C:N with time might have explained the low and constant GGE. This study highlighted the importance of predator in increasing the yield of the system (particularly at high C:N_S) and as a mechanism of regulation of the

system. N regeneration was similar in both bacteria alone and bacteria and grazer experiments, suggesting that flagellates were inefficient in the transfer of N. However, *P. vestita* enhanced the bacterial utilisation of DOC by grazing on *V. natriegens*. Finally, this experiment indicates that the common assumption, particularly in mathematical models, of constant C:N_P (for example the C:N of bacteria) has to be taken with caution, along with the importance of the C:N ratio of the substrate.

- CHAPTER 5 -

The role of N or Si limitation on diatom DOM production and subsequent microbial community dynamics.

5.1 Introduction

Dissolved organic matter (DOM) is an important substrate for bacterial growth in aquatic ecosystems (Azam & Hodson 1977, Azam et al. 1994, Hedges et al. 1997). The breakdown of this matter supports bacterial biomass increase (which may in turn be grazed by heterotrophic predators) with a fraction being regenerated in inorganic form. DOM may result from riverine (Meybeck 1993, Hedges et al. 1997) or atmospheric (Buatmenard et al. 1989, Hedges & Keil 1995) input, from anthropogenic sources (Jickells 1998) or from micro- and meso-zooplankton grazing (Strom et al. 1997, Nagata 2000). In addition to these various sources, extracellular release of DOM by phytoplankton contributes significantly to the DOM pool (Carlson & Hansell 2002).

Diatoms (Bacillariophyceae) are a particularly important component of the phytoplankton, contributing 20 ~ 25 % of the global net primary production (Werner 1977). Their requirement for silicon makes them most prevalent in coastal waters (Conley & Malone 1992), where a spring bloom of diatoms is often the major feature of the annual cycle of plankton succession. The large magnitude of the spring diatom bloom suggests that factors governing the quantity and quality of the DOM produced in the nutrient limited senescent phase of this event are likely to be of critical importance to the subsequent abundance, composition and productivity of the bacterial communities of coastal waters.

Nutrient stress has been shown to stimulate this DOM excretion by phytoplankton (Lancelot & Billen 1984). Moreover, as nutrient stress influences the chemical composition of phytoplankton (Droop 1968, Caperon & Meyer 1972, Sakshaug et al. 1983, Obernosterer & Herndl 1995), it may influence the quality and quantity of DOM released (Sondergaard et al. 2000, Puddu et al. 2003, Grossart & Simon 2007). For diatoms, Mykkestad (1974) and Mykkestad et al. (1977) demonstrated that glucans (polymers of glucose) are common storage products, which provide a suite of bioavailable compounds (Hama et al. 2004). Glucans only accumulate in diatoms when growth is suboptimal (Mykkestad 1974), a result that may be influenced by the form of nutrient limitation (N-or Si-limited) experienced by the population (Gilpin et al. 2004). For example, enhanced glucan concentrations are found under N-limitation. Under silicate limiting conditions, however, silicification processes within diatom cells are compromised leading to weaker cell walls and potentially “leaky” cells (Martin-Jézéquel et al. 2000), with the potential for enhanced DOM release (Maestrini & Granéli 1991). Hence, the form of nutrient limitation is likely to influence both quantity and quality of DOM produced by diatoms and, hypothetically, the resultant growth and composition of the bacterial populations utilising the organic matter released.

In Scottish and other temperate waters, *Skeletonema* sp. are commonly the dominant diatoms within the spring bloom (Tett 1992, Fehling et al. 2006). Hence, the period marking their cessation and decline might be expected to introduce significant DOM loading within coastal waters. The inorganic N:Si ratio within these and other temperate waters is often close to one (Conley & Malone 1992, Fehling et al. 2006) with both nutrients having the potential to be the limiting factor that arrests the spring diatom bloom. The field study detailed in Chapter 3, highlighted that the N:Si ratio varied with time and hence could have an influence on the microbial, particularly the phytoplankton, dynamics. In addition, the field study of Scottish coastal water (Chapter

3) indicated that the phytoplankton was the dominant source of organic carbon. This study, therefore, investigated the effect of N- and Si- limitation on the extracellular release of DOM by the important diatom *Skeletonema costatum* and the utilisation of this material by natural bacterial assemblages collected from coastal waters. Using time course incubations within laboratory microcosms we studied bacterial abundance, production and taxonomic composition. As grazing pressure from heterotrophic flagellates is also important in controlling bacterial populations (Caron 2000, Caron et al. 2000), DOM addition experiments were conducted in the presence (whole seawater) and absence (1µm-screened seawater) of these grazers.

5.2 Experimental design and additional methods

The experiments were conducted in two stages (Fig. 5.01). Initially, cultures of *Skeletonema costatum* were grown to nutrient limited stationary phase under either N or Si limitation. The DOM produced was collected by ultra-filtration and then subsequently quantified and characterised by HTOCO (high temperature catalytic oxidation) and HPLC (high performance liquid chromatography) respectively. Secondly this harvested DOM was added to freshly collected samples of natural seawater. The subsequent changes in bacterial abundance, production and community composition, produced in response to DOM generated by N- or Si-limited *S. costatum*, were measured. DOM addition experiments were conducted using natural seawater with or without screening for grazers, in order to generate conditions representing natural and reduced density of *in situ* grazing flagellates.

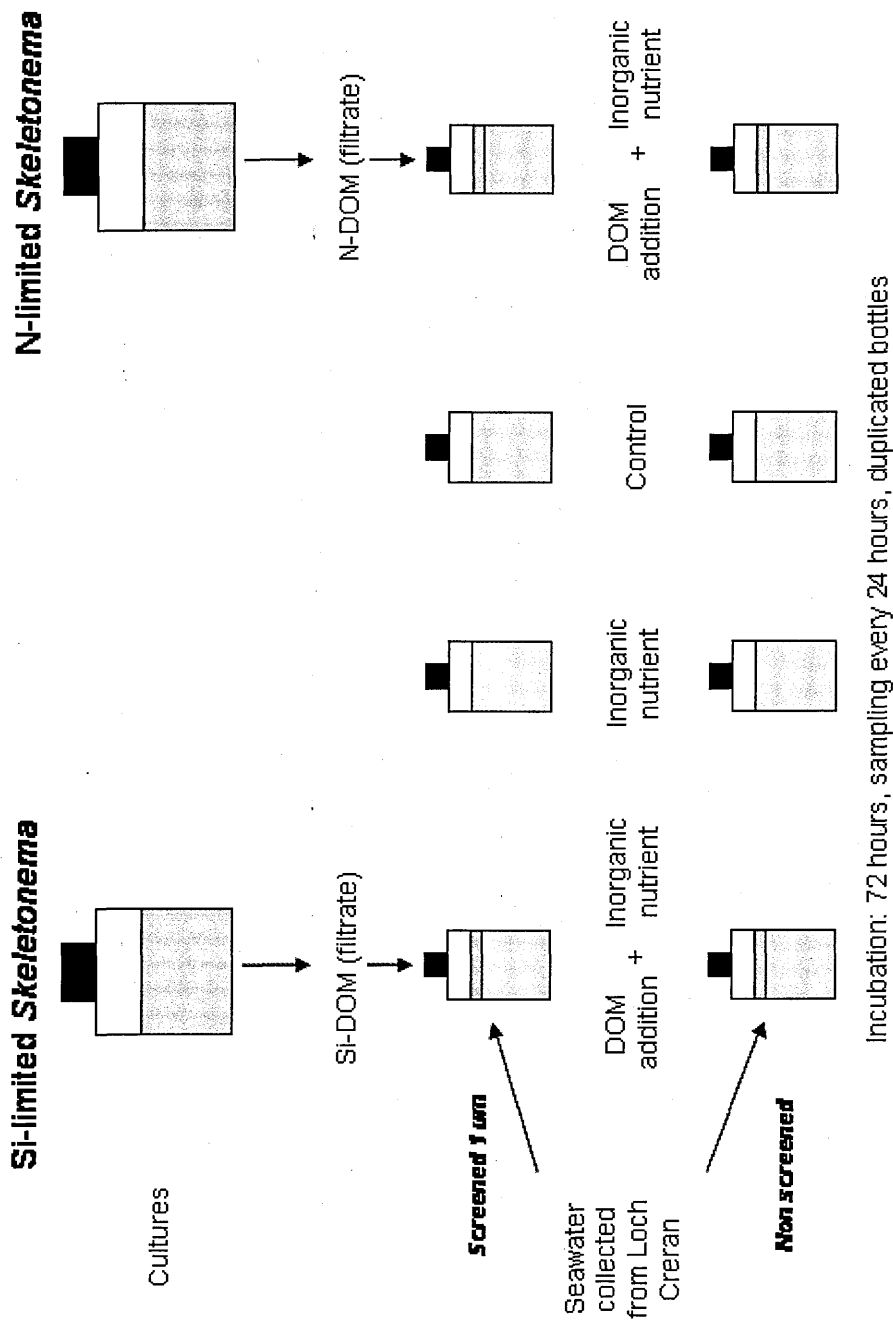


Figure 5.01 Synoptic of the experimental design

5.2.1 *Skeletonema costatum* cultures: generation of DOM.

Axenic cultures of *Skeletonema costatum* (Cleve 1897), previously isolated from the field work station (C5) of Loch Creran (CCAP, 1077/9), were grown in 10 litre polycarbonate carboys containing autoclaved filtered seawater (8 litre) with a slightly modified f/2 medium (Table 5.01). EDTA was excluded to avoid additional sources of DOC. Cells were maintained at 15°C under a 12/12 light/dark photo-cycle and a photon flux density of 160 $\mu\text{E m}^{-2} \text{ s}^{-1}$. All experimental equipment was both acid washed and steam-sterilised (121° C; 15 minutes) prior to use. All experiments were conducted in duplicate.

In order to generate DOM characteristic of N- and Si- limited phytoplankton, different initial concentrations of inorganic nitrate and silicate were used. To achieve N limitation, a N:Si ratio of 1:3 (40 μM and 120 μM , respectively, see Table 5.01) was used with silicate limited diatoms being produced through growth on a N:Si ratio of 3:1 (120 μM and 40 μM , respectively). *S. costatum* growth was followed by the daily collection of samples for the determination of chlorophyll a (chl *a*). 30 ml of samples were analysed as described in section 2.1.3.3, in Chapter 2.

Table 5.01. Composition of experimental media for *Skeletonema costatum* cultures.

	Concentration	Volume added (ml)		Final conc. (μM)	
		Si-lim.	N-lim.	Si-lim.	N-lim.
NO ₃	0.1 M	12	4	120	40
Si(OH) ₄	0.1 M	4	12	40	120
PO ₄	5.565 mg/L	10	10	37	37
Vitamins	f/2	10	10	f/2	f/2
Trace metals	f/2	1	1	f/2	f/2

5.2.2 DOM harvest and ultrafiltration

DOM generated within the cultures was harvested during nutrient limited stationary phase (after 14 days growth in both nutrient regimes). Cultures were filtered by gravity through a pre-ashed GF/F filter (Whatman, 0.7 μm nominal porosity; Nayar & Chou 2003), and DOM containing filtrate was collected in acid cleaned conical flasks. DOM was subject to tangential filtration (Schleicher & Schuell, Germany), using a 1 kDa membrane, to enable determination of the DOC and DON content of the low molecular weight (LMW < 1kDa) and high molecular weight (HMW > 1kDa) extracts of the phytoplankton DOM (Saunders 1976).

5.2.3 DOM addition experiment

Seawater was collected from 10 m with a Niskin bottle from Loch Creran (station C5), then pre-screened with a 160- μm mesh net to remove macro-zooplankton and the screened sample stored in a Nalgene carboy during its rapid return to the laboratory (Fig. 5.01). Half of the seawater sample was then filtered by gravity through GF/F filters to reduce grazers (screened seawater) with the remaining half being used as collected (non-screened seawater).

DOM generated from both N- and Si-limited *Skeletonema costatum* cultures was added to duplicated one litre flasks containing the Loch Creran seawater, either screened or non screened (see Table 5.02). Inorganic nutrients as nitrate, silicate and phosphorus (10 μM , 10 μM , 1 μM , respectively) were added with the DOM to prevent inadvertent inorganic nutrient limitation of bacterial growth. This enabled the results of this study to be related to the lability and influence of DOM in inorganic nutrient replete conditions only. To provide control comparisons, two additional treatments (using both

screened and non-screened Creran seawater) received inorganic nutrients only (no DOM) or no nutrient addition (inorganic or organic). In the following section, treatments are referred as (1) “Si-DOM” for treatments receiving DOM from Si-limited phytoplankton cultures, (2) “N-DOM” for treatments receiving DOM from N-limited phytoplankton cultures, (3) “Inorganic” for inorganic nutrient additions alone and (4) “control” when no additions were made. All experimental and sampling equipment was acid cleaned and rinsed with milli-Q water to prevent any organic carbon or nitrogen contamination. Flasks were incubated for 72 hours at 15°C on a 12/12 hr light/dark cycle under a photon flux density of $160 \mu\text{moles.m}^{-2}.\text{s}^{-1}$, and were sampled, after gentle shaking, every 24 hours.

Table 5.02. Experimental set up with additions of phytoplankton-produced DOM and inorganic nutrients.

	Si-limited produced DOM	N-limited produced DOM	NO_3^- (μM)	PO_4^{3-} (μM)	Si(OH)_4 (μM)
Screened	1:9 ^a	-	10	1	10
	-	1:9 ^a	10	1	10
	-	-	10	1	10
	-	-	-	-	-
Non-screened	1:9 ^a	-	10	1	10
	-	1:9 ^a	10	1	10
	-	-	10	1	10
	-	-	-	-	-

^a volume:volume addition

5.2.4 Sample analyses

Inorganic nutrient concentrations in the cultures were monitored every two days and were analysed according to section 2.1.3.1, in Chapter 2. DOC and DON samples were collected every two days in the cultures and for each time point of the time course incubation, and analysed according to section 2.1.3.2, Chapter 2. The extraction of the carbohydrates from the DOM harvested and the subsequent liquid chromatography

analyses are detailed in section 2.3.2.2 in Chapter 2. Enumeration of HNAN was performed according to section 2.1.4.1.2 in Chapter 2. Bacterial abundance (BA) and bacterial production (BP) were measured following the methods described in sections 2.1.4.1.4 and 2.1.4.2, Chapter 2, respectively. Finally, Bacterioplankton community structure was analysed by FISH following the method described in section 2.4.2 in Chapter 2.

5.3 Results

5.3.1 Phytoplankton generated DOM

Both *Skeletonma costatum* cultures reached maximum abundance between day seven and eight with chl *a* concentrations of 0.16 and 0.10 mg chl *a* equivalent m⁻³ for Si and N limitation respectively. In Si-limited cultures, diatom cells continued to take up nitrate when silicate was exhausted, whereas silicate uptake ceased with N exhaustion in N limited cultures (Fig. 5.02). When the *S. costatum* cultures were harvested, at day 14, DOC concentrations were 319 µM and 239 µM for Si- and N-limited cultures respectively. DON concentrations were 35 and 29 µM for Si- and N-limited cultures respectively.

5.3.2 Characterisation of the DOM produced by *S.costatum*

Following tangential filtration, the relative proportion of LMW and HMW compounds were similar in the DOM generated in both nutrient regimes. The majority

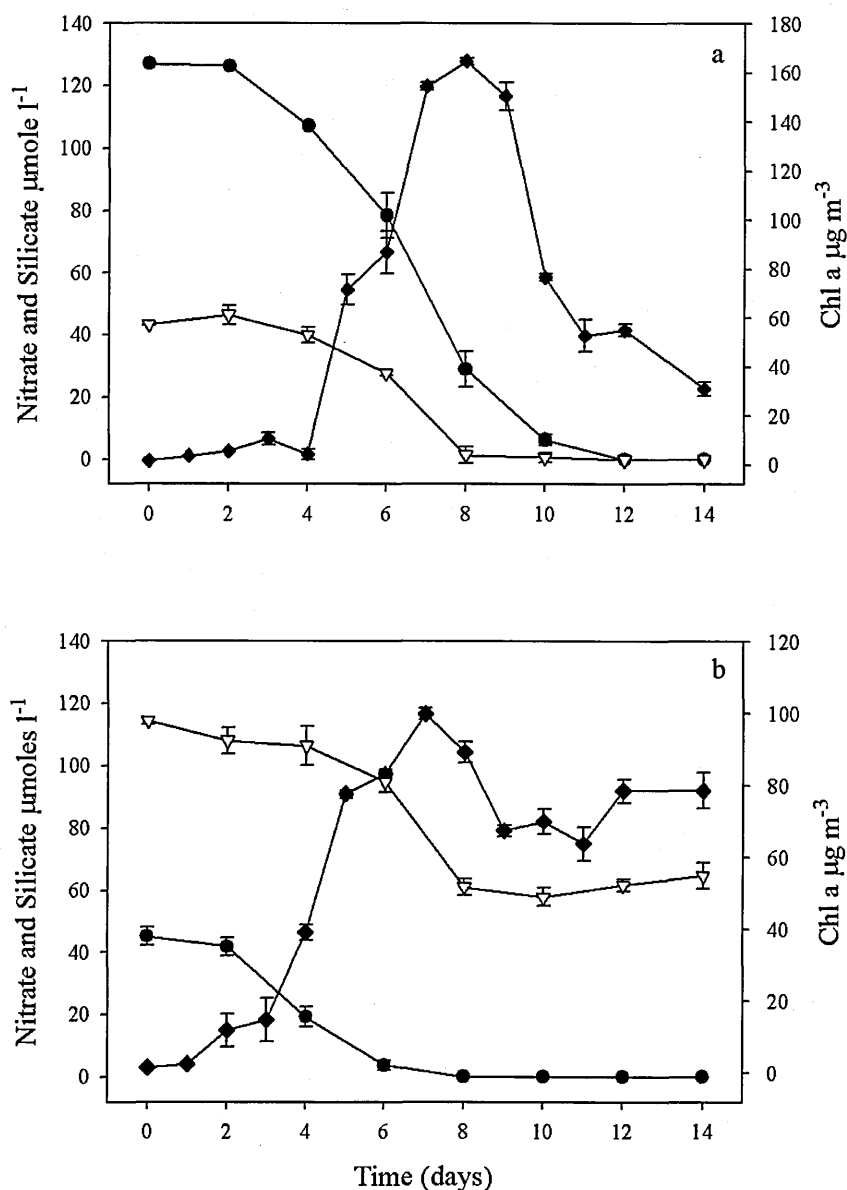


Figure 5.02. Chlorophyll a (♦) nitrate (●) and silicate (▽) concentrations in both (a) N-limited (b) Si-limited cultures of *Skeletonema costatum*.

of DOC produced was composed of LMW compounds (Table 5.03), with 72% and 68% for Si- and N-limited cultures, respectively. A similar pattern was observed for DON with 59 and 69% of the organic matter being LMW compounds for Si- and N-limited cultures respectively.

Table 5.03. Percentage of LMW and HMW in DOC and DON in inoculums, and C:N ratio for each molecular weight fraction.

	DOC		DON		C:N	
	LMW	HMW	LMW	HMW	LMW	HMW
N-DOM	68.5	31.5	69.3	30.7	6.9	7.2
Si-DOM	72.6	27.4	59.5	40.5	5.0	3.3

Notwithstanding the similarities in the bulk composition measurements above, chromatography of DOM extracts produced by Si- and N-limited phytoplankton revealed differences in their composition. During analysis of mono- and di-saccharides (sugar-like compounds) in the Si-limited DOM on a Polyamine II column, a distinct peak with an absorption maximum at 210 nm eluted at retention time 18.7 min (Fig. 5.03 a and b). In contrast, no signals were recorded in N-limited DOM under the chromatographic conditions used. Chromatography of these carbohydrate extracts on a PL-GFC column revealed differences in molecular weight composition. Figure 5.03-c indicates that the N-limited extracts were dominated by two high molecular-weight components, as demonstrated by the refractive index (RI) signals that eluted at 10.5 and 13.6 min. Using a plot of log M_r of standards versus retention time, the relative molecular mass of these peaks were estimated to be >1,400,000 and 330,000 Da respectively. Since the first peak eluted in the void volume of this column, this peak could represent one or more high molecular weight species, the mass of which were unresolvable under the conditions used, yet dominating the composition of these extracts. DOM produced by Si-limited phytoplankton displayed a similar peak profile except for the distinctly reduced signal of the peak defining the void volume (Fig. 5.03-d).

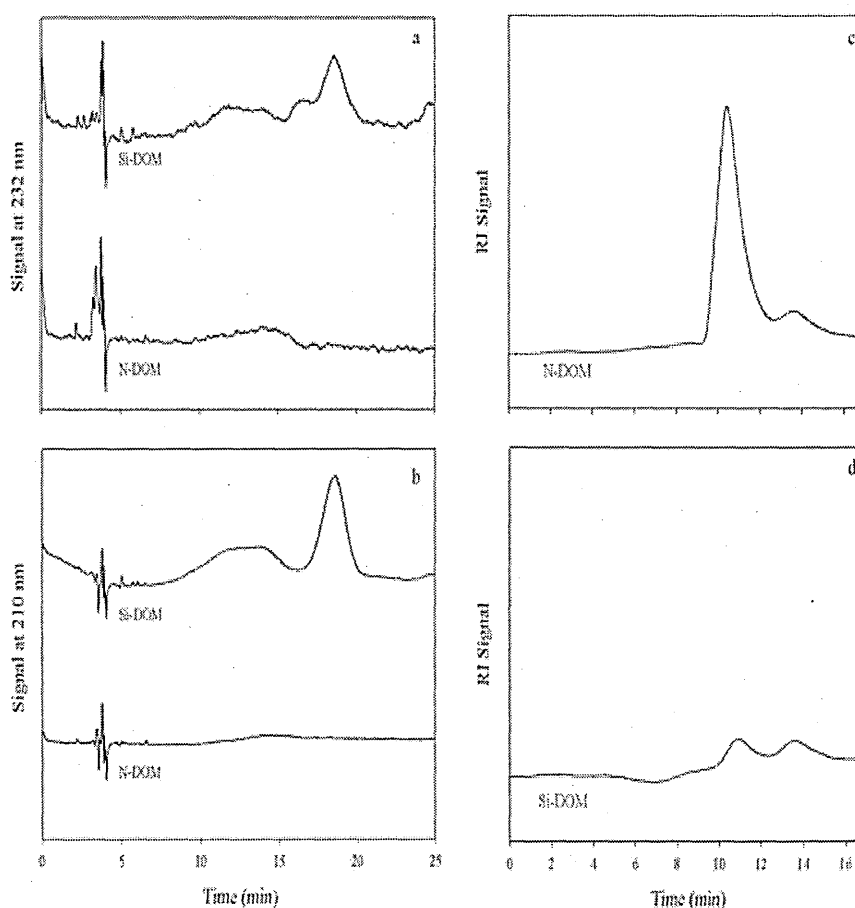


Figure 5.03. HPLC chromatograms of extract from Si-limited *S. costatum* with Polyamine II column (a, b) or PL GFC column (d) and extract from N-limited *S. costatum* with PL GFC column (c).

5.3.3 DOM addition experiments: initial DOM concentrations

Seawater sampled from Loch Creran (station C5) had *in situ* DOC and DON concentrations of 120 μM and 13 μM , respectively, as can be observed from the t_{zero} concentrations of the control and inorganic treatments (Fig. 5.04). DOM harvested from both Si- and N-limited *S. costatum* was added separately to the Loch Creran seawater to obtain similar concentrations of DOC and DON in the Si-DOM and the N-DOM incubations. These concentrations supplemented the background DOC and DON to give t_{zero} concentrations of 200 – 230 μM C and 16 - 17 μM N (Fig. 5.04).

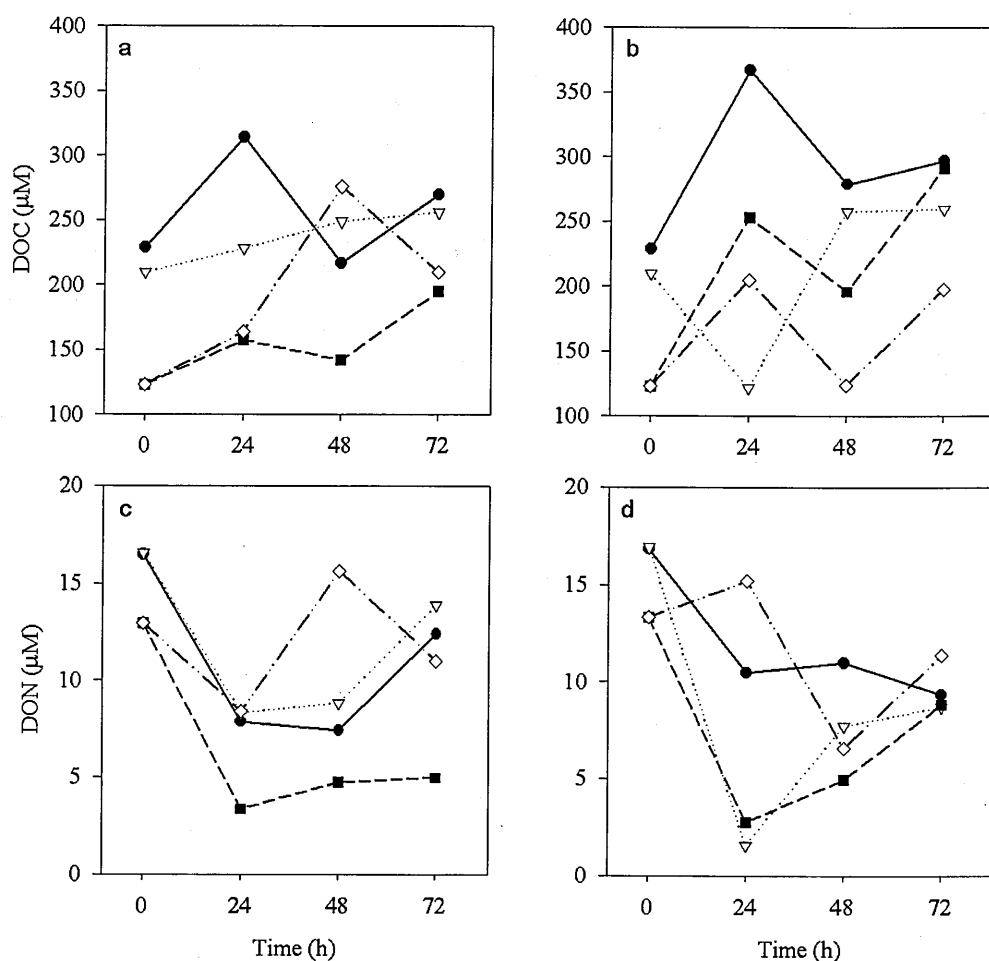


Figure 5.04. Dissolved organic carbon (DOC) and nitrogen (DON) concentrations in screened (a, c) and non-screened (b, d) seawater subject to Si-DOM (●), N-DOM (▽), Inorganic (■) and control (◇) treatments. No error bars are shown as calculated standard error was smaller than analytical accuracy ($C \pm 11 \mu\text{M}$, $N \pm 0.9 \mu\text{M}$).

5.3.4 Changes in dissolved organic C and N during the addition experiments

Considerable variability in DOC concentrations was evident over the experimental time course. However, in the screened treatments, DOC concentrations generally increased from the beginning to end of the experiments (one-way ANOVA, $p < 0.01$), but with no significant differences between treatments (Fig. 5.04-a). In contrast, no significant differences in DOC concentration either with time or treatments were

found in the non-screened incubations (Fig. 5.04-c). In contrast to DOC, DON concentrations decreased in screened seawater until 48h (t_{48}) in DOM addition bottles (1-way ANOVA, $p < 0.05$) then increased between 48 and 72 (t_{72}) hours (Fig. 5.04-b). In unfiltered treatments, DON concentrations were not statistically different between treatments.

5.3.5 Bacterial abundance and production

Marked differences in BA were evident within and between screened treatments. BA more than doubled between t_{zero} and t_{24} in all cases (one-way ANOVA, $p < 0.01$). However, only in the Si-DOM treatment did BA continue to increase subsequently (Fig. 5.05-a), with BA being significantly greater at t_{48} and t_{72} in this treatment (one-way ANOVA, $p < 0.01$). BP in the screened Si-DOM treatment increased from an average of 6.8 to 11.4 $\mu\text{gC L}^{-1} \text{d}^{-1}$ (Fig. 5.05-c) and was significantly higher than in the other three treatments (one-way ANOVA, $p < 0.01$), in this case for the whole experiment including t_{24} . In contrast, while BP remained finite in the N-DOM treatment, it decreased in a similar manner to the inorganic and control treatments (no statistical difference between treatments, one-way ANOVA, $p > 0.05$).

In the non-screened treatments a different pattern was observed. BA initially increased, but to a lower abundance ($\sim 15 \times 10^8$ cf. 20×10^8 cells L^{-1}) at t_{24} (Fig. 5.05-b). Then, although, BA in the Si-DOM treatment exceeded the others at time t_{48} and t_{72} , no significant differences were evident between treatments. BP decreased from t_{zero} to t_{72} (Fig. 5.05-d) with time in all treatments (one-way ANOVA, $p < 0.01$) from 10.5 to ~ 4 $\mu\text{g C L}^{-1} \text{d}^{-1}$. While no significant differences were found between treatments, at t_{24} both BA and BP were lowest in the non-screened Si-DOM treatment.

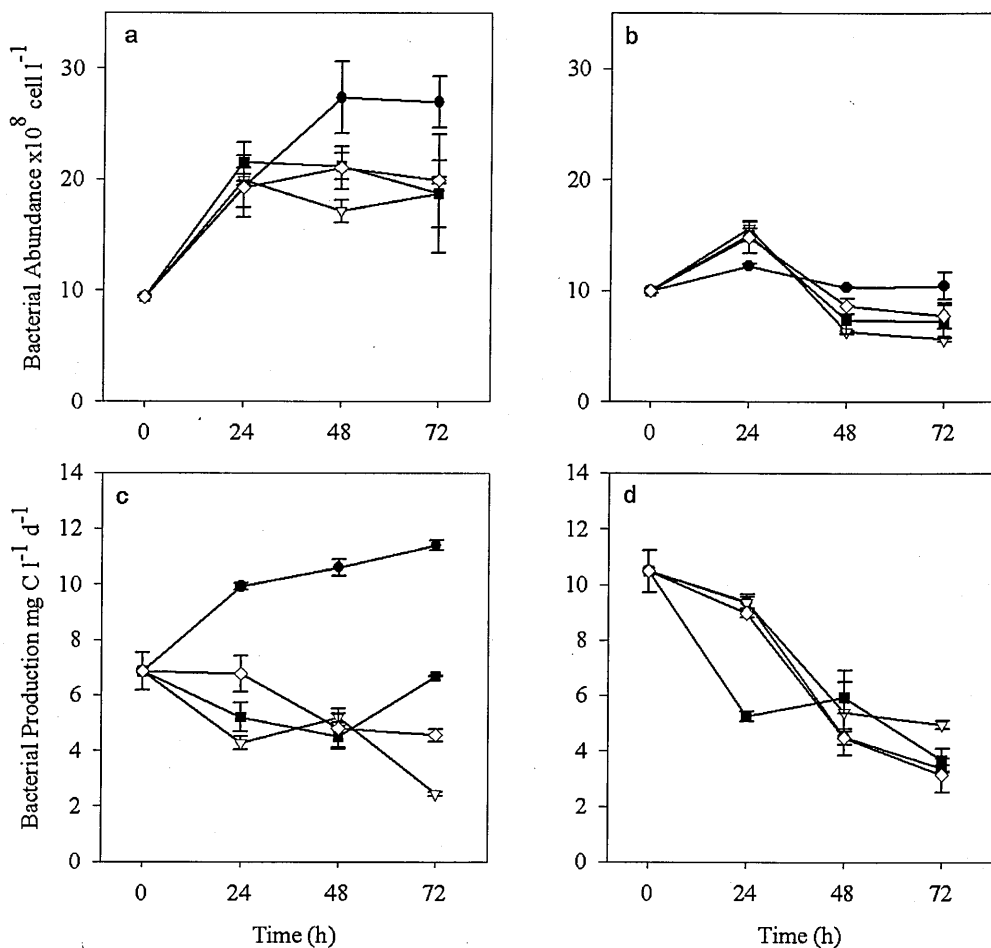


Figure 5.05. Bacterial abundance (BA) and production (BP) in screened (a, c) and non-screened (b, d) seawater subject to Si-DOM (●), N-DOM (▽), Inorganic (■) and control (◇) treatments. Error bars are standard error.

5.3.6 Bacterial community composition

FISH and epifluorescence microscopy were conducted on screened treatments only and revealed bacterial community composition to be governed by substrate quality (Fig. 5.06). Unfortunately, analysis of the t_{zero} samples was unsuccessful, however as all treatments came from the same water sample, we can hence assume that the t_{zero} community composition was identical for all experiments. At t_{24} , *γ-proteobacteria*

dominated the community in all four treatments (Fig. 5.06), comprising ~60% (N-DOM treatment) to ~40% (control) of the bacterial community as determined by DAPI. *β-proteobacteria* were also present in Si-DOM and N-DOM treatments at t_{24} but not in inorganic and control treatments.

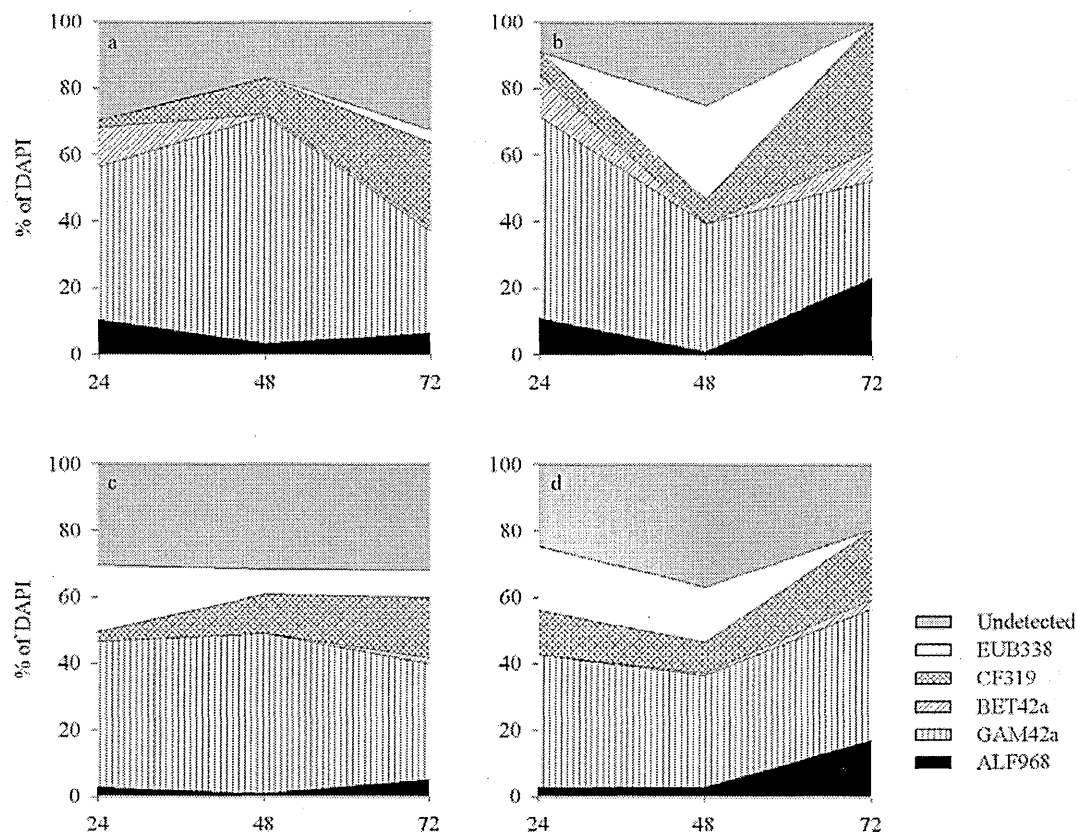


Figure 5.06. Time course of bacteria taxonomic composition in screened seawater samples subject to (a) Si-DOM, (b) N-DOM, (c) Inorganic, and (d) control treatments. Legend refers to Eubacteria (EUB338), Cytophaga-Flavobacterium or CFB (CF319), *β-proteobacteria* (BET42a), *γ-proteobacteria* (GAM42a) and *α-proteobacteria* (ALF968).

As incubation continued, the dominance of *γ-proteobacteria* increased markedly in the Si-DOM treatment at t_{48} reaching 68.4 % of the total community (Fig. 5.06-a). In contrast, this group remained approximately constant in the inorganic treatment (Fig. 5.06-c) and decreased in the N-DOM and control treatment at t_{48} (Fig. 5.06-b and -d, respectively), with an unlabelled group of bacteria showing a transient increase at this time. In addition, *β-proteobacteria* almost disappeared from the community at t_{48} . All

four treatments also exhibited, relative to t_{24} , a higher proportion of CFB like bacteria at both t_{48} and more markedly at t_{72} , reaching 45 % in the N-DOM treatment, against 17-25 % in other treatments. α -proteobacteria and β -proteobacteria were also present in all treatments at this point with the N-DOM and control treatments exhibiting significant increase of the α -proteobacteria between t_{48} and t_{72} (23 % and 17 %, respectively). Hence, while γ -Proteobacteria remained the dominant group, bacterial community compositions at t_{72} were different from those found at t_{24} changing from γ -proteobacteria domination to a balance between γ -proteobacteria, the CFB group and α -proteobacteria but with treatment specific differences in the proportions of each group.

5.3.7 HNAN grazer abundance

Due to the plasticity of nanoflagellate cells and their resultant ability to pass through membrane pores much smaller than their typical diameter, screening of the Loch Creran seawater reduced rather than eliminated HNAN grazers within the screened incubations. Hence, in screened water, HNAN abundance was initially approximately half that in the non-screened water (Fig 5.07).

Subsequently, in screened incubations, HNAN abundance increased until t_{48} in the Si-DOM, N-DOM and inorganic treatments (Fig. 5.07-a), with concentrations at that time ranging from 1×10^6 to $\sim 3 \times 10^6$ cells L^{-1} . Abundance then decreased between t_{48} and t_{72} . In non screened treatments HNAN abundance also increased within the first 24 hours, then ceased for Si-DOM, inorganic and control, but continued to increase up to t_{48} in the N-DOM treatment (Fig. 5.07-b).

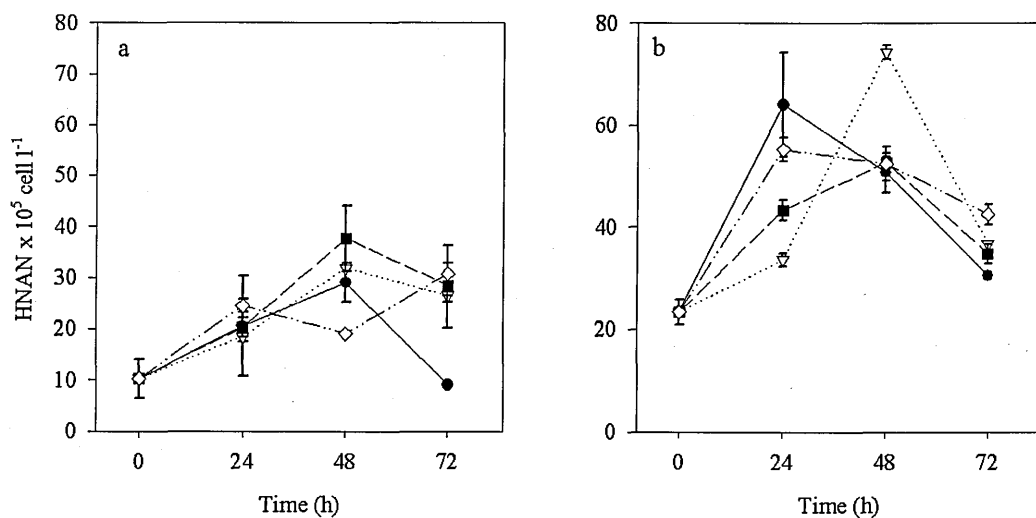


Figure 5.07. HNAN abundance for (a) screened, and (b) non-screened seawater subject to Si-DOM (●), N-DOM (▽), Inorganic (■) and control (◇) treatments. Error bars are \pm S.E. from duplicates.

5.4 Discussion

S. costatum was chosen as the source of DOM for the study as it frequently dominates the phytoplankton biomass during the spring bloom in Loch Creran and hence may be the single most important source of autochthonous DOM in these and other coastal waters. The particular strain studied had previously been isolated from Loch Creran from where the bacterial population, to which the DOM was added, was obtained. While it would have been possible, through nutrient manipulation, to generate N or Si limitation of natural seawater samples rather than to generate this DOM from laboratory cultures, the subsequent characterisation, quantification and manipulation of DOM concentrations would have been extremely problematic.

5.4.1 DOM production

DOM was harvested after the marked decrease in the *S. costatum* biomass subsequent to its Si-limited nutrient limited peak. Such a major decrease in diatom biomass under Si limitation is consistent with results from *S. costatum* dominated mesocosms (Gilpin et al. 2004) and is similar to a rapid decrease in abundance we observed previously in Si-limited (but not P-limited) cultures of the diatom *Pseudo-nitzschia seriata* which often dominates the summer diatom community in Scottish waters (Fehling 2004).

DOC concentrations were greater in DOM harvested from Si than in N-limited cultures (319 and 239 μM respectively). However, if the DOC and DON produced (by day 14) was normalised to peak chl *a* concentrations (160 vs. 100 $\mu\text{g chl } a \text{ m}^{-3}$), there was relatively more DOC (23.1 cf. 31.4 $\text{mg C (mg chl } a)^{-1}$) and DON (2.9 cf 4.5 $\text{mg N (mg chl } a)^{-1}$) generated per unit biomass under N rather than Si limitation.

Reduced silicification under Si-limitation (Brzezinski 1985, Martin-Jézéquel et al. 2000) leading to weaker and leaky cell walls, would be expected to contribute to enhanced release of intracellular organic compounds. However, the N-limited *S. costatum*, population that maintained a high biomass in stationary phase still generated relatively more DOM, perhaps indicating that much of the Si-limited diatom biomass was converted to particulate rather than dissolved organic matter.

As nitrate was exhausted in both Si- and N-limited conditions we can calculate the percentage conversion of nitrate to DON by *S. costatum*; this gives 29% and 72% for Si- and N-limited cultures respectively, indicating an extremely efficient conversion of N from inorganic to organic form in N-limited conditions. Hence, while one might expect that further DOM production by N-limited *S. costatum* would occur with time, as

the biomass declined, the capacity for further DON production at least would be quite limited.

Within the DOM, Si- and N-limitation generated similar proportions of LMW and HMW material. Within the DOC pool, both treatments exhibited ~70% LMW and ~30% HMW. For DON, the LMW fraction was again dominant although somewhat less so under Si than N limitation (~60% LMW cf. ~70% LMW). The relative proportion of LMW and HMW matter has been suggested to be an important index of biological availability of DOM, with LMW material being expected to be more labile (Saunders 1976). Although this conceptual model is still widely accepted other authors have demonstrated that HMW compounds may also be rapidly utilised by bacteria (Tranvik 1990, Arnosti et al. 1994, Amon et al. 2001). For example, Amon et al. (1996) reported higher rates of bacterial growth and respiration in HMW incubations than those with LMW incubations and concluded that the traditional model of DOM degradation does not appear to apply to the bulk of natural DOM. As we added the same amount of total DOM (as a combination of LMW and HMW material) to our microcosm experiments we cannot comment on the relative bioavailability of the two fractions. However, as discussed below, the greater bacterial productivity observed in the treatments receiving Si-DOM suggests differences in bio-availability that cannot be related to size alone. As measuring the bulk concentration of the DOM and in each molecular weight fraction is not sufficient to describe the differences in biological activity observed, we investigated the qualitative aspect of the DOM used in our experiment.

Qualitative characterisation of the carbohydrate extracts from the DOM confirmed that the form of nutrient limitation influenced the form of both LMW and HMW DOM pools. Analysis indicated that *S. costatum* produced small, sugar-like, compounds when Si-limited. In contrast, larger (≥ 1400 kDa) compounds were produced by N-limited *S. costatum*, consistent with the greater biomass specific production of

DOM in this case. Silicate uptake stopped with N exhaustion in N-limited cultures. However, as it has been demonstrated elsewhere (Davidson & Gurney 1999) phytoplankton may continue to fix C for some time under N-limitation leading to the production of these large carbohydrates. As the total organic matter content of the LMW and HMW pools were similar in both Si- and N-limited treatments, other compounds, unidentified in this carbohydrate based analysis, such as lipids and proteins will have contributed to the organic matter pools.

Hence, organic matter composition, rather than size seems to be the key factor in governing the growth of the natural assemblage of bacteria. If labile organic matter favours the growth of bacteria, larger and more complex compounds can, in contrast, exert a limiting effect on bacterial growth by their inherent refractory nature. This feature is common and has been reported for exopolysaccharides produced by micro-algae (Aluwihare & Repeta 1999) and bacteria (Anton et al. 1988, Bejar et al. 1996, Ogawa et al. 1999, Gutierrez et al. 2007).

5.4.2 Changes in bulk bacterial properties

The similarity in BA and BP between inorganic and control treatments in both screened and unscreened conditions indicates that the addition of inorganic nutrients did not enhance bacterial growth and hence that inorganic nutrients were not limiting the community. This is in agreement with the observations made in Chapter 3 and inorganic nutrient replete conditions in Loch Creran are discussed in Chapter 6. However, the most striking result of the study was the sustained elevation of BP and BA in the screened Si-DOM treatment in comparison to the values recorded at t_{zero} and to all other treatments throughout the experiment. As both the Si- and N-DOM treatments were also supplemented with inorganic nutrients one may conclude that bacterial metabolism was

limited by the availability of organic matter, but that only the Si-DOM was sufficiently labile to alleviate this limitation. This is consistent with the characterisation of the DOM above in indicating that there were sufficient quantities of biologically available compounds within the Si-DOM to support elevated bacterial abundance and productivity over at least 72 hours.

Somewhat, in contrast with these results, is the lack of a clear pattern of changes in DOM (and in particular DOC) concentrations in the experiments. As these experiments were conducted in carefully controlled laboratory conditions, albeit with natural seawater samples, this illustrates the difficulty in drawing conclusions on DOM-driven microbial dynamics simply from changes in concentrations of organic matter.

As noted above, the conceptual model of LMW DOM being more biologically labile is widely held. However, N-DOM contained relatively more LMW DON and similar proportions of LMW DOC than Si-DOM. Hence, at least for DOM production by *S. costatum*, the biochemical composition of the DOM is of much greater importance than its size spectrum in governing bacterial community response.

5.4.3 Effect on bacterial community composition

The bacterial community structure also demonstrated differences that were related to the form of nutrient addition. The failure of the t_{zero} FISH samples means any analysis of the community shifts between t_{zero} and t_{24} was impossible. However, as noted above this community was identical for all experiments, and from our previous work on bacterial communities in Scottish and Norwegian coastal waters (Davidson et al. 2007, Hart et al. unpublished data) it is likely that the bacterial assemblage present at t_{zero} were dominated by the α -*proteobacteria*, the γ -*proteobacteria* and the CFB group.

At t_{24} dominance of the γ -*proteobacteria* lineage in all our experiments is consistent with its ability to respond rapidly to inorganic nutrients (Ferguson & Sunda 1984), thus out competing the α -*proteobacteria*, which comprised only a comparatively small fraction of the t_{24} communities (ranging from 2.7% to 10.8% in the control and N-DOM treatments respectively). Puddu et al. (2003) observed a similar trend when a bacterial assemblage was amended with glucose or extracellular organic carbon (EOC) produced in balanced growth conditions by the diatom *Cylindrotheca closterium* (Ehrenberg).

However, the most significant finding between t_{zero} and t_{24} was the increased BP in the Si-DOM treatment. Again this is almost certainly attributed to the dominant γ -*proteobacteria* rapidly utilising the labile components of the Si-DOM, although a similar or greater percentage of γ -*proteobacteria* was present in the N-limited treatment. This is consistent with the hypothesis of Fuchs et al. (2000) that the fast growing members of the γ -*proteobacteria* fill the niche of typical r-strategists, which rapidly exploit extra nutrients when they become available.

As the incubations continued, between t_{24} and t_{48} , the relative abundance (from 46.1% to 68.4%) and absolute abundance (data not shown) of the γ -*proteobacteria* increased significantly only in the Si-DOM treatment. In contrast, their contribution to the bacterial assemblages in the N-DOM reduced significantly, and both the inorganic and control treatments showed no significant change (Fig. 5). Again, these shifts in the bacterial assemblage are consistent with observed continued elevation in BP in the Si-DOM treatment. The lack of significant increase in BA suggests that C demand fulfilled an increase in cell activity (and biomass) rather than cell division.

At t_{72} in the N-DOM treatment there was a significant increase in the relative and absolute abundances of the CFB group (up to 45.2% of the DAPI stained prokaryotic community) and to a lesser extent the α -*proteobacteria* (up to 23.1%) but

without a concomitant increase in BA or BP. The relative CFB group abundance also increased in the Si-DOM treatment, coinciding with a reduction in the absolute and relative abundances of the *γ-proteobacteria*. Similar, but less pronounced, changes occurred in the inorganic and control treatments. Members of the CFB group and *α-proteobacteria* are adept at utilising exudates from algal productivity (Rooney-Varga et al. 2005) including DMSP, amino acids, and for the CFB group, more complex macromolecules. Members of the CFB group are also more abundant in media rich in particulate organic matter or complex macromolecules (DeLong et al. 1993)(DeLong et al. 1993) and probably figure as k-strategists (Kirchman 2000, Puddu et al. 2003). Hence, although the *γ-proteobacteria* remained dominant within all the treatments, at t_{72} the CFB group may also be active utilising HMW DOM unavailable to other groups.

In the case of the *α-proteobacteria* their increased abundance at t_{72} may result from reduced competition from *γ-proteobacteria* as its dominance declined as the labile components of the DOM available to this group were depleted. This was consistent with the minimal increase in *α-proteobacteria* in the Si-DOM treatment, suggesting that they remain out-competed by the active *γ-proteobacteria*.

These results therefore suggest that *γ-proteobacteria* may have an initial competitive advantage when DOM exudates from Si-limited diatoms are available. However, competitive advantage for resources evolved rapidly with time, most probably through the depletion of labile organic C leading the bacterial consortia to adapt to the remaining source of less labile C, and hence leading to changes in bacterial community composition. Kirchman et al. (2004) described similar evolution of bacterial properties, such ectoenzymatic activities, with the modifications of the DOM, hence suggesting that DOM may modify the relative abundance of bacterial subgroups, which in turn, affect the DOM pool.

5.4.4 Effect of grazers in modulating the bacterial community response

Heterotrophic nano-flagellates had a strong effect on bacterial density markedly reducing the increase in BA at t_{24} and causing a decrease in net BP with time in all non screened treatments. While not statistically different, both the BA and BP at t_{24} in the Si-limited non screened treatments were the lowest recorded at that time. Considering that these quantities were statistically significantly higher in the screened Si-DOM treatments this suggests selective grazing on the actively growing γ -proteobacteria in this treatment.

In all except the inorganic treatment the difference between t_{zero} and peak HNAN density was markedly greater in the non screened treatments (Fig. 5.07), consistent with lower abundance of bacteria in this scenario. This is indicative of the major role played by heterotrophic grazers in controlling bacterial populations (Gasol 1994, Strom 2000). However, as HNAN density in the Si-DOM non screened treatment was not significantly greater than the others these results may suggest that much of the extra productivity in these conditions is recycled within the microbial loop.

5.5 Conclusion

In conclusion, the results of this short-term incubation experiment indicate that DOM produced by N-limited *S. costatum* is less available to bacterial uptake than it is for exudates from Si-limited cultures. These two different forms of DOM also affect the taxonomic composition of the bacterioplankton community, promoting the subclass of γ -proteobacteria. The comparison of screened and non-screened conditions highlighted the role of grazers in controlling the bacterial population and their indirect effect on C dynamic in the microbial food web. This is in agreement with the observations made in Chapter 3 and 4. If we carefully extrapolate these results to the natural ecosystem of

Loch Creran, this experiment provides insightful information on the effect of a shift from N to Si limitation in coastal water. Indeed, as suggested in Chapter 3, phytoplankton is the main source of production of DOM in Loch Creran and the Firth of Lorn. The high value of the N:Si ratio observed during the spring suggested a relative lack of Si that would further indicate a non biologically available character of the DOM produced (as suggested in this Chapter with N-limitation). This also gives insights into DOC accumulation, which has been observed to occur in temperate coastal waters from the end of the spring bloom through the summer months (see also Chapter 3, Cauwet et al. 2002), as nutrient stress may lead to changes in bacterial consortia composition that are not able to degrade DOM present in the environment. The marked differences, for BP in particular, between treatments suggest that the role of inorganic nutrient limitation (further discussed in Chapter 6) and other factors governing phytoplankton physiology, and hence metabolic state, requires more detailed study of the role of autochthonous DOM as a driver for marine microbial community dynamics in the context of marine coastal systems.

- CHAPTER 6 -

Factors limiting microbial communities from LY1 and Loch

Creran

6.1 Rational

The control of bacterial populations in marine system is often summarised as comprising bottom-up (nutrient resource) and/or top-down (grazing) mechanisms. It has long been recognised that bacterial growth depends on the availability of organic carbon as the source of energy (LeB Williams 1981). In marine systems, this organic carbon may come from phytoplankton or macroalgae exudation (LeB Williams 2000, Nagata 2000), viral lysis (Proctor & Fuhrman 1992) or terrestrial sources (Cauwet et al. 2002). In addition, recent studies have highlighted the importance of inorganic nutrients as limiting factors of bacterial production (Caron 1994, Kirchman 1994) and that low inorganic N or P concentrations, as well as organic nutrients, may have a limiting effect on bacterial dynamics. The use of inorganic nutrients by bacteria also indicates that bacterioplankton compete with phytoplankton for these resources (Bratbak & Thingstad 1985, Caron et al. 2000). In the light of the recognition of phytoplankton-bacteria competition for inorganic nutrient, studies of the form of nutrient limiting bacterial production in coastal waters are surprisingly rare.

Caron et al. (2000) investigated the degree of limitation of the bacterial and phytoplankton populations between the productive coastal region of George Bank (USA) and the oligotrophic system of the Sargasso Sea (south Bermuda). In order to determine the limiting factor of the bacteria and phytoplankton growth, these authors designed an experiment where they added inorganic (N and P) or organic nutrients

(glucose) to samples from each location. These authors found that the factors limiting the productivity were different for the contrasting locations. At George Bank glucose provoked an increase in productivity, whereas in the Sargasso Sea, the addition of inorganic N and P did increase bacterial production and suggested that low inorganic nutrient concentrations were limiting the microbial community productivity.

The motivation for the experiment described in this Chapter, originated from observations made during summer 2004, where elevated BP and BA were noted during August but were not associated with elevated concentrations of DOC and DON. This experiment therefore investigated if the form of limitation (inorganic or organic) of the microbial community may explain these observations. The present experiment adopted a similar approach to that of Caron et al. (2000) using inorganic and organic nutrient additions to natural assemblages from LY1 and FF stations. In addition it includes the comparison of filtered and unfiltered samples allowing to assess the importance of the grazing pressure on bacterial population dynamics.

6.2 Experimental design and additional methods

6.2.1 Collection of seawater

Water samples were collected from a depth of 10 m with a 10 L Niskin bottle, on the 22/08/05 and 26/08/05 from LY1 and FF respectively (see map, Fig. 2.01, Chapter 2). Water samples were immediately pre-screened through a 160 μ m mesh net to remove large grazers and were kept in the dark at ambient temperature until further processing in the laboratory.

6.2.2 Experimental set up

In the laboratory, water samples from LY1 and FF were filtered through GF/C filters (Whatman) to remove nano-planktonic grazers or used as unfiltered samples. 1.5 L of media was placed in 2 L conical flasks supplemented with: (1) inorganic nitrogen (NaNO_3) and phosphorus (NaHPO_4), (2) organic C as glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) and (3) a control that received no additions (Table 6.01). All experimental flasks were duplicated and incubated in a controlled-temperature room at 13°C with a light intensity of $100 \mu\text{moles m}^{-2}$ on a 15-9 hours light-dark cycle. All treatments were sampled to determine bacterial abundance (BA) and production (BP), as well as HNAN and PNAN abundances. Sampling was conducted at the time of additions (t_{zero}) then after 24 and 48 hours of incubation. A sub-samples of each treatments was taken to determine the primary production (PP) and extra cellular organic carbon (EOC) after 24 and 48 hours.

Table 6.01. Nutrient additions

	LY1			FF		
	Inorganic N and P $\mu\text{moles L}^{-1}$	Organic C $\mu\text{moles L}^{-1}$	Control	Inorganic N and P $\mu\text{moles L}^{-1}$	Organic C $\mu\text{moles L}^{-1}$	Control
Whole seawater	N: 15 P: 1	18.7	-	N: 15 P: 1	18.7	-
Screened water ($<1 \mu\text{m}$)	N: 15 P: 1	18.7	-	N: 15 P: 1	18.7	-

BA was determined using DAPI staining and epifluorescence microscopy (see section 2.4.1.4, chapter 2) and BP was measured by incorporation of radiolabelled thymidine (see section 2.4.2, chapter 2). PNAN and HNAN abundances were determined by DAPI staining and counted using epifluorescence microscopy as described in section 2.4.1.2, chapter 2. Primary production and extracellular organic carbon were determined according to section 2.3.4 in Chapter 2.

6.3 Results

6.3.1 Water column characteristics at LY1 and FF on the day of each experiment

The water column structure, chemical and biological variables indicated that LY1 and FF were under similar trophic regimes (Table 6.02); however some differences existed. Both stations exhibited similar salinity and temperature. All inorganic nutrients except DSi (dissolved inorganic silicate) and ammonium were present at higher concentrations at LY1. While POC, PON and chl *a* were similar in magnitude, concentrations were higher at FF than LY1. BP at FF was approximately twice that found at LY1 (25 v.s.11 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ at FF and LY1, respectively). However, abundances of HNAN, PNAN and heterotrophic bacteria were similar at both sites.

Table 6.02. Environmental variables, and bacterial, HNAN and PNAN abundances in experimental water samples collected for the experiment

Parameters		LY1	FF
HNAN	$\times 10^5 \text{ cell L}^{-1}$	15.9	14.2
PNAN	$\times 10^5 \text{ cell L}^{-1}$	163.5	160.1
POC	$\mu\text{g C L}^{-1}$	177.0	260.0
PON	$\mu\text{g C L}^{-1}$	27.0	40.0
Chl <i>a</i>	$\mu\text{g L}^{-1}$	1.6	2.4
BA	$\times 10^9 \text{ cell L}^{-1}$	2.0	2.1
BP	$\mu\text{g C L}^{-1} \text{ d}^{-1}$	11.3	25.7
DOC	$\mu\text{moles L}^{-1}$	157.9	119.5
DON	$\mu\text{moles L}^{-1}$	6.3	9.6
DIN	$\mu\text{moles L}^{-1}$	0.5	0.1
DSi	$\mu\text{moles L}^{-1}$	4.7	5.3
DIP	$\mu\text{moles L}^{-1}$	0.6	0.3
Ammonium	$\mu\text{moles L}^{-1}$	1.2	1.8
Temperature	$^{\circ}\text{C}$	13.5	13.9
Salinity		33.3	32.7

It is important to note that the parameters at t_{zero} presented hereafter were determined for each of the three treatments (organic, inorganic and control) in both filtered and

unfiltered water but were averaged for the clarity of the data presentation. Although both sites demonstrated a balanced particulate C to N ratio (~ 6.5 at both sites) in terms of Redfield proportion (Redfield 1963), inorganic N:P ratios (0.8 and 0.3 for LY1 and FF, respectively) suggested a strong N depletion at both sites. This background data set provided useful information on the status of these two sites.

6.3.2 LY1 experiment

Primary production (PP) during time course incubations of the unscreened (containing phytoplankton) ranged from 40 to 52 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ (Fig. 6.01-a). PP increased significantly (1-way ANOVA, $p < 0.05$) when glucose was added but both N+P and glucose additions exhibited lower PP than the control. EOC produced during the experiment increased with time and was greater when glucose was added (Fig. 6.01-b) with values reaching 3 $\mu\text{g L}^{-1} \text{ d}^{-1}$. However, EOC was not detected in the control due to high blank recorded at the start of the experiment.

BA ranged from 13 to 33 $\times 10^8 \text{ cell L}^{-1}$ with maximal values attained during the experiment supplemented with glucose (Fig. 6.02-a). In filtered water, BA increased in all treatments, with significantly higher ($p < 0.05$) BA for the glucose amendment treatment at t_{24} and t_{48} . However, the N+P treatment was not statistically different from control. In unfiltered water, BA increased at t_{24} in all treatments with highest abundances found following glucose additions. At t_{48} , BA decreased in N+P and the control, whereas glucose treatments produced similar levels to t_{24} .

BP covered a range of values from 17 to 73 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ and varied greatly for the different treatments (Fig. 6.03-a). Similarly to BA, BP increased significantly (1-way ANOVA, $p\text{-value} < 0.01$) at t_{24} and t_{48} in filtered water, with the greatest increase observed when glucose was added ($+ 22 \mu\text{g C L}^{-1} \text{ d}^{-1}$ compared to N+P and the control

at t_{48}). However, BP in the treatment with N+P additions was not significantly different from the control. In unfiltered seawater, BP decreased in N+P and control during time

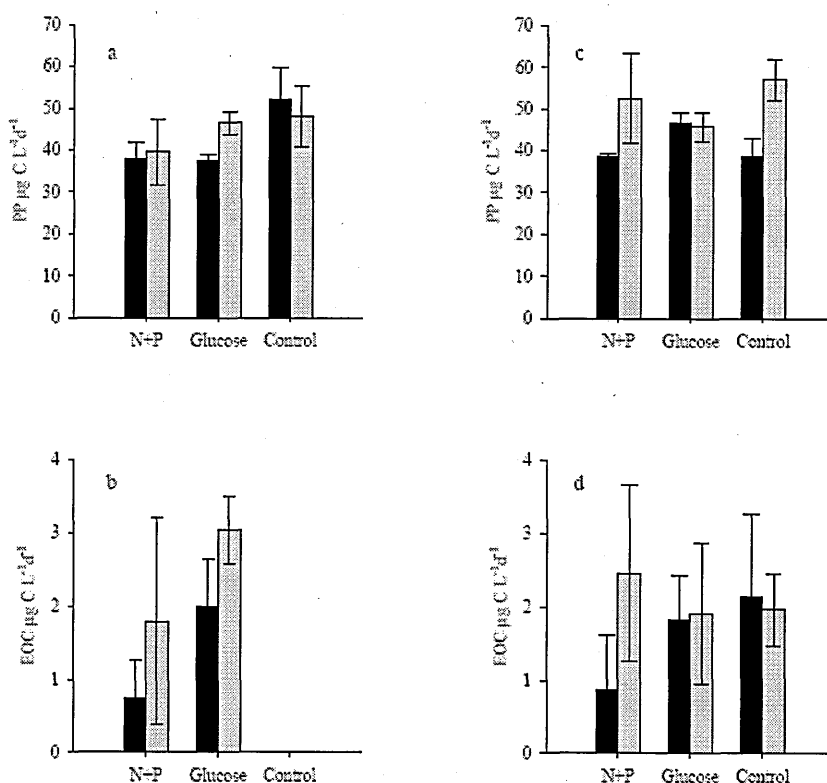


Figure 6.01. Primary production (PP) and extracellular organic carbon (EOC) for the three treatments (indicated on the x axis) at LY1 (a, b) and FF (c, d) in non-screened water. Data presented at t_{24} (black) and t_{48} (gray). Error bars are s.e.

course incubations (Fig. 6.03-b). Glucose amended treatments remained at similar levels of BP between 24 and 48 hours.

HNAN numbers ranged from 2 to $30 \times 10^5 \text{ cell L}^{-1}$. In contrast to PNAN, filtration at $1 \mu\text{m}$ removed 30 to 75 % of HNAN. Abundances of HNAN in filtered water remained similar (N+P and glucose), or decreased between t_{zero} and t_{48} (Fig. 6.04-a). For unfiltered treatments, only flasks amended with glucose increased significantly (1-way ANOVA, $p < 0.05$) during time course incubations (Fig. 6.04-b).

PNAN abundance varied from 10 to $135 \times 10^5 \text{ cell L}^{-1}$ with highest values found in unfiltered water (Fig. 6.05-a). The screening process removed approximately 90 % of

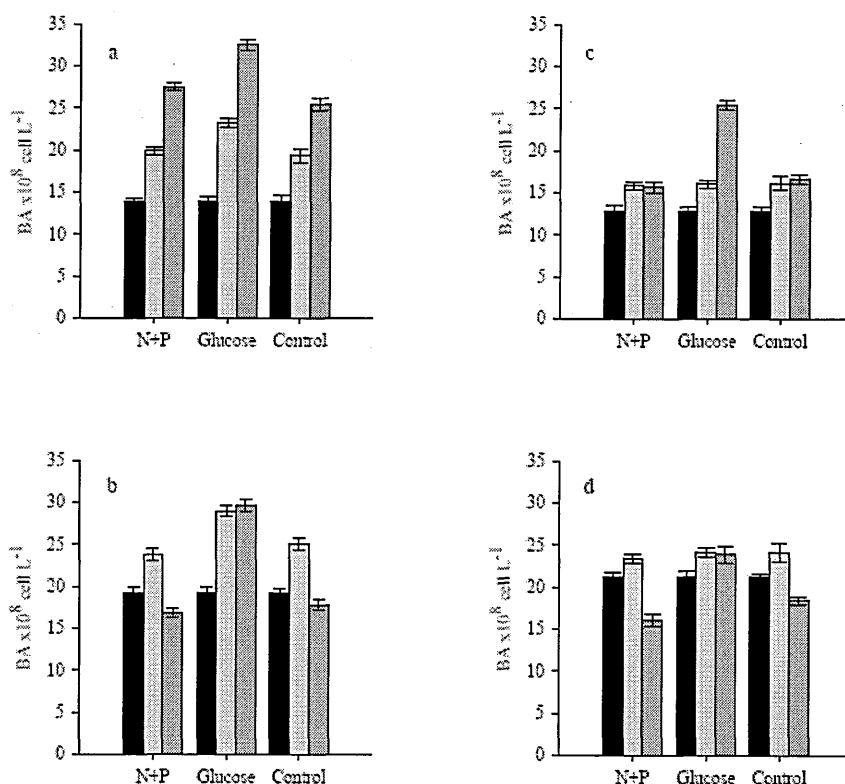


Figure 6.02. Bacterial abundance (BA) for the three treatments at LY1 (a,b) and FF (c, d) experiments in filtered (a, c) and unfiltered (b, d). Values presented for t_{zero} (black), t_{24} (light gray) and t_{48} (dark gray). Error bars are s.e.

the PNAN, in all treatments. In filtered water, N+P additions stimulated the growth of PNAN with abundance increasing about four fold between t_{zero} and t_{48} . However, PNAN abundance at t_{48} in glucose and control treatments did not differ significantly from t_{zero} . Similarly to filtered water, N+P additions for the unfiltered experiment provoked an increase in PNAN abundance (Fig.6.05-b), whilst it decreased in glucose additions and remained approximately constant in the control.

6.3.3 FF experiment

PP in non-screened samples collected from the FF station ranged between 38

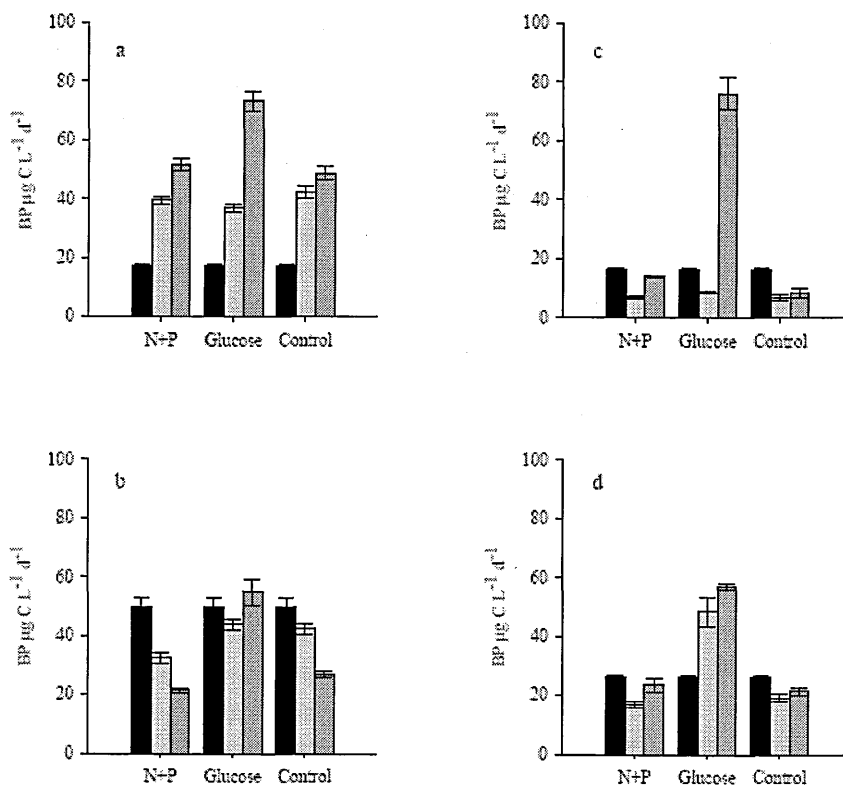


Figure 6.03. Bacterial production (BP) for the three treatments at LY1 (a,b) and FF (c, d) experiments in filtered (a, c) and unfiltered (b, d). Values presented for t_{zero} (black), t_{24} (light gray) and t_{48} (dark gray). Error bars are s.e.

and $57 \mu\text{g C L}^{-1} \text{d}^{-1}$. BP increased in N+P and control treatments but no changes were observed for the glucose addition. EOC also increased between t_{24} and t_{48} for the N+P addition but was not significantly different from controls. EOC were found in similar range in control and glucose addition treatment. Overall, treatments were not significantly different from each other (Fig. 6.01-c and 6.01-d).

BA ranged between 13 and $25 \times 10^8 \text{ cell L}^{-1}$ (Fig. 6.02-c). In filtered water, BA had increased (1-way ANOVA, $p < 0.05$) by approximately $3 \times 10^8 \text{ cell L}^{-1}$ in all treatments at t_{24} . However, a further increase in BA (of $9.4 \times 10^8 \text{ cell L}^{-1}$) was only noticeable for the glucose amended treatment between t_{24} and t_{48} (1-way ANOVA, $p < 0.05$).

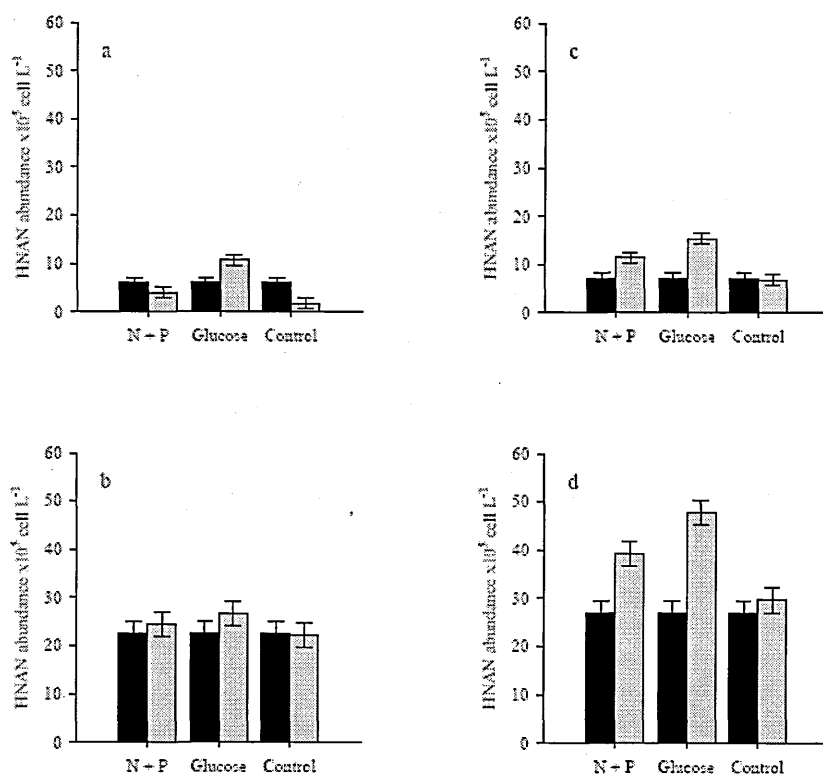


Figure 6.04. HNAN abundance (x10⁵ cell L⁻¹) for the three treatments (indicated on the x axis) at LY1 (a,b) and FF (c, d) experiments in filtered (a, c) and unfiltered (b, d). Black bars are t_{zero} and gray bars are t₄₈. Error bars are s.e.

In unfiltered samples, BA was generally higher (1-way ANOVA, $p < 0.05$) than in filtered water (Fig. 6.02-d). BA increased for all treatments at t₂₄, then decreased significantly (1-way ANOVA, $p < 0.05$) for N+P and the control. However, BA was maintained at the same level in the glucose-supplemented treatment.

Similarly to LY1, BP ranged from 17 to 73 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ in incubations with water from FF. In filtered water, BP exhibited a significant decrease ($p < 0.05$) of 8-10 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ at t₂₄ (Fig. 6.03-c). These increases in BP were concomitant to those observed with BA. Then, BP increased significantly (1-way ANOVA, $p < 0.05$) in N+P and glucose amended treatments (7 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ and 68 $\mu\text{g C L}^{-1} \text{ d}^{-1}$, respectively). In unfiltered water, BP decreased at t₂₄ in N+P and control treatment whereas it increased

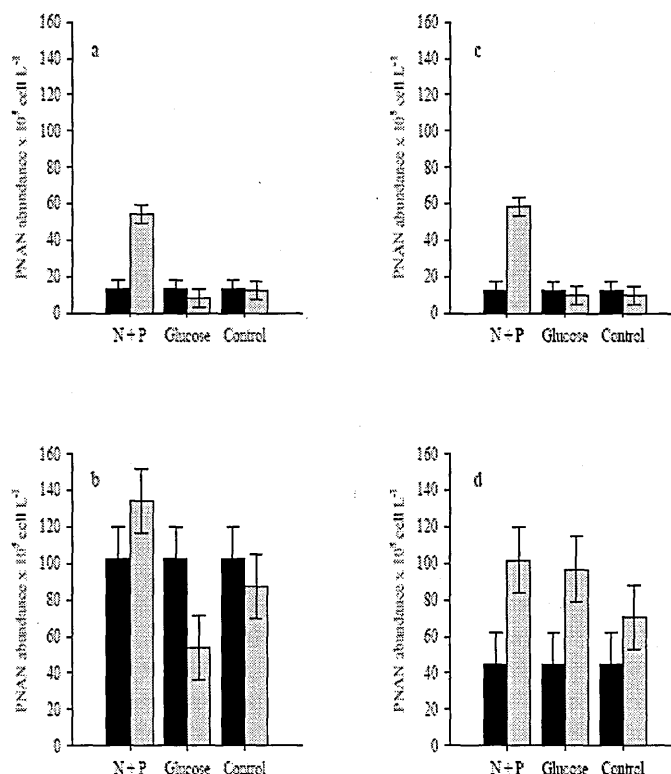


Figure 6.05. PNAN abundance ($\times 10^5$ cell L⁻¹) for the three treatments (indicated on the x axis) at LY1 (a,b) and FF (c, d) experiments in filtered (a, c) and unfiltered (b, d). Black bars are t_{zero} and gray bars are t₄₈. Error bars are s.e.

significantly (1-way ANOVA, $p < 0.05$) on glucose addition treatment (Fig. 6.03-d). Subsequently, BP increased significantly only in glucose amended water, to reach 56 $\mu\text{g C L}^{-1} \text{ d}^{-1}$ at t₄₈.

HNAN abundances varied between 5 and 47 $\times 10^8$ cell L⁻¹, comparable to LY1. HNAN abundance increased (between t_{zero} and t₄₈) when nutrients (N+P and glucose) were added to filtered water (Fig. 6.04-c), with significantly higher numbers (1-way ANOVA, $p\text{-value} < 0.05$) obtained when glucose was added (8×10^5 cell L⁻¹, as compared to 4×10^5 cell L⁻¹ in N+P treatment). A similar pattern was observed in unfiltered samples (Fig. 6.04-d), with abundances of HNAN two to nine times higher than those found in filtered water.

PNAN densities were comparable to those found for the LY1 experiments, ranging from 9 to 102×10^5 cell L⁻¹. In filtered water, only N+P addition provoked an increase of PNAN abundances (Fig. 6.05-c). The control and glucose treatments remained unchanged between t_{zero} and t_{48} (Fig. 6.05-c). However, PNAN numbers increased for all treatments when samples were unfiltered (Fig. 6.05-d). These increases were significant for N+P and glucose additions, but overall there were no significant differences between treatments.

6.4 Discussion

This set of experiments demonstrated the differences between LY1 and FF in terms of the response of their microbial communities to amendments of inorganic or organic nutrients. The factors controlling bacterial populations has been extensively studied in open oceans (Sherr et al. 1986, Sherr & Sherr 1987, Sherr et al. 1992, Kirchman 2000, Strom 2000, Davidson et al. 2007) in the detriment of the coastal regions. Understanding the dynamics of bacterioplankton on small time and space scales is still a challenge, as it represents a very active component of the whole microbial food web. Marine heterotrophic bacteria are known to utilise DOM for their growth (LeB Williams 1981, Azam et al. 1983), but also have the ability to take up inorganic nutrients at low concentrations (Kirchman 1994, Wheeler et al. 1996). The ecological significance of this latter behaviour resides in the fact that they can therefore enter into competition with phytoplankton for inorganic resources (Rivkin & Anderson 1997, Cotner & Biddanda 2002).

Phytoplankton growth, as determined by the PP (Fig. 6.01), showed little difference between treatments and no difference between the two locations studied. Indeed, PP measured for N+P additions was similar to the control and organic supplemented samples (Fig. 6.01-a and 6.01-c). This might suggest that phytoplankton

responded similarly in the two different environments and, that PP and phytoplankton biomass were nutrient replete in this experiment. In fact, when water samples were collected for this experiment (August 2005), the phytoplankton community was dominated by diatoms (see Chapter 3, section 3.7.4, including the *Pseudo nitzschia seriata* group, *Leptocylindrus danicus*, *Chaetoceros* sp and *Rhizosolenia* sp) and DSi concentrations were 4.7 and 5.3 $\mu\text{mole L}^{-1}$ (Table 6.02) for LY1 and FF stations, respectively. These findings are in agreement with those of Egge and Aknes (1992) who found the dominance of diatom to occur when Si concentrations were greater than 2 $\mu\text{moles L}^{-1}$.

In this experiment primary producers were exposed to the same constant light intensity ($\sim 100 \mu\text{moles m}^{-2} \text{s}^{-1}$), regardless of the treatment. Light intensity is a crucial factor in photosynthetic processes (Rhee & Gotham 1981) and may have been limiting. Differences in light intensity between the laboratory and natural conditions could have triggered important photosynthetic activity therefore masking the potential effects of nutrient additions on PP. A combined effect of nutrients and light would have resulted in greater PP than other treatments (glucose and control); however, PP in the N+P amendment treatment was not statistically different from the control (no addition) for both LY1 and the FF experiments.

The healthy nutritional conditions of the phytoplankton community were in agreement with the EOC concentrations observed, which represented a very minor fraction of the PP (ranging between 0 to 7 %). These low levels of EOC reflected the good health of phytoplankton, in contrast to the high EOC concentrations produced from stressed phytoplankton (Mykkestad 1995, Granum et al. 2002). In chapter 5, a marine diatom, *Skeletonema costatum*, was grown under nutrient limitation (N and Si) and produced labile DOC under Si limitation that stimulated the growth of bacteria. In the experiment detailed here, the Si-replete conditions are likely to be associated with

low concentrations of DOC produced by the phytoplankton. Furthermore, this DOC did not enhance BP, which remained at its lowest level in control whereas PP was at similar levels in all other treatments. In addition, the concentration of EOC varied between 0 and $0.25 \mu\text{moles L}^{-1}$, which probably made an insignificant contribution to the DOC concentrations measured at t_{zero} (Table 6.02); it was therefore probably not significant in accounting for the variation of BP observed.

The ratio of autotrophic production (PP) to bacterial production (BP) ranged from 1.25 to 5 for all non-screened treatments, suggesting that autotrophy was exceeding heterotrophy in these time course incubations. This further corroborated the replete nutritional conditions of phytoplankton and suggested that bacteria may have been already limited before the start of the experiment.

BP initially increased for all LY1 treatments, but with greater production observed at t_{48} for the glucose addition. These observations were concomitant with an increase in BA and implied the active growth of the bacterial population in the experimental conditions, showing uptake of C (BP) and cell division (BA) in all treatments.

In contrast, only FF experiments with filtered water exhibited a marked stimulation of BP (Fig. 6.03-c) and BA (Fig. 6.03-c) following glucose additions. These observations suggested different types of limitation of the bacterioplankton between LY1 and FF. First of all, the availability of organic carbon was clearly limiting for the FF experiments (potentially of more terrigenous origin; see chapter 3) as only a readily available C source triggered the growth of bacteria. In contrast, the results suggested that organic carbon was initially available in sufficient concentrations at LY1 for use by bacteria. Glucose addition then promoted further production at t_{48} (approximately of $20 \mu\text{g C L}^{-1} \text{ d}^{-1}$ compare to N+P and control). As DOC concentrations were higher at LY1

than FF (Table 6.02), this denoted that a part of the DOC pool at LY1 was potentially labile.

Nutrient resources were available in the filtrate of the control treatment at LY1, as evidenced by the increase in BA (Fig. 6.02-a and 6.02-c) and BP (Fig. 6.03-a and 6.03-c) observed in filtered water. However, it is unclear whether this substrate was present before the filtration processes, or if it is a consequence of it. Filtration of seawater samples might have had three types of effects:

(1) the removal of resources that come from phytoplankton or grazing leading to lower bacterial growth. This was unlikely here as even in the presence of phytoplankton cells the production of EOC would not have supported significant bacterial growth. Assuming a bacterial abundance to C conversion factor of $30.2 \text{ fg C cell}^{-1}$ (Fukuda et al. 1998) and a bacterial growth efficiency of 0.27 (Gasol & del Giorgio 2000), both typical of coastal water, the EOC produced would have supported the production of approximately $0.27 \times 10^8 \text{ cell L}^{-1}$. This is 10 and 40 times less than the cell number increase that was observed in the non-screened control treatments at FF and LY1, respectively;

(2) the disruption of fragile cells leading to the nutrient enrichment in the experiment. This feature might have been possible at LY1 (where BP and BA increased in all filtered treatments) but as water samples were processed in the same way for FF and LY1, and phytoplankton biomass was greater at FF site, the same pattern (or an even greater increase) would have been expected in FF experiments;

(3) the removal of grazers. This is the most likely explanation to the response observed in the bacterioplankton as LY1 and FF might have experienced different grazing pressures leading to the differences observed. However, the filtration process probably removed some of the initial bacterial standing stock (diminished BA at t_{zero} in filtered water compared to unfiltered water). Glucose additions that promoted the

growth of bacteria in LY1 and FF experiment, in filtered samples, may have occurred in unfiltered water but the differences would have been minimised by the presence of grazers.

HNAN could have been, in fact, inefficient grazers, as they did not transform bacterial biomass ingested into HNAN biomass. The latter scenario was suggested by other authors (Goldman et al. 1987, Strom et al. 1997) and is also discussed in Chapter 4. In the LY1 experiment, in unfiltered water, BP decreased for the N+P and the control treatment at t_{24} potentially due to active grazing concomitant to cell division, as shown by the increase of BA at t_{24} . Although, this demonstrates an active grazing of bacteria, HNAN abundances remained unchanged during the LY1 experiment (Fig. 6.04-b). This, then, suggests that other grazers as well as HNAN may have been active. Dinoflagellates and ciliates were present at the time of the experiment (see Chapter 3) and are known to feed on bacteria (Sherr, 1991).

The release of grazing pressure through screening of the water might have helped the bacteria to freely utilise the organic resources available, which suggested that an active grazing of the bacterioplankton was occurring at LY1 before the start of the experiment. In contrast, grazing was not active at first at the FF station but was stimulated subsequent to bacterial growth, promoted by glucose additions. In fact, the increases in bacterial activity in unfiltered water from station FF (Fig. 6.03-d) were concomitant with a significant increase of HNAN in the glucose additions. When glucose was added to the water from LY1 it promoted further production (compared to the N+P treatment and control) which was reflected in the maintained level of BP (Fig. 6.03-b) as well as number between t_{24} and t_{48} (Fig. 6.02-b).

Finally, in common with phytoplankton, inorganic nutrient limitation of the bacterial populations in LY1 and FF experiments was not observed, as the addition of inorganic N and P did not stimulate greater production or numbers compared to the

controls. However, inorganic nutrient addition did stimulate the growth of PNAN in filtered samples for both LY1 and FF experiments.

Inorganic nutrients present in natural water at the time of the experiment (see Table 6.02 and Chapter 3) were greater at FF than LY1. In unfiltered samples, these inorganic nutrients might have been responsible for the increase in PNAN abundance observed, in all cases, for the FF experiment (Fig. 6.05-d). In comparison, in the LY1 experiment, the decrease of PNAN for the glucose addition might suggest that they were out-competed by bacteria for the use of the low level of inorganic nutrient concentration, but when inorganic nutrients were added (N+P addition), PNAN did indeed increase.

6.5 Conclusion

In summary, this experiment revealed an existing difference in the nutritional status of the microbial population between LY1 and FF. The sufficient N and P at both stations similarly stimulated PP. Organic nutrient addition suggested that the bacterial population were C limited at the FF site. Although initial availability of organic resources to bacteria was possible at LY1, the bacterial growth became C limited. It appears that only a small fraction of DOC was available. In addition, the bacterial growth at LY1 was masked by an intense but potentially inefficient, grazing pressure. More broadly, it appears that the control of bacterial population is far more complex than bottom-up and top-down regulation. This experiment highlighted the fact that the metabolic state of the bacterioplankton, and their grazers, may influence the response to nutrient enrichment. This experiment also demonstrated that spatial variability of microbial dynamics exists in coastal waters and that this spatial variability may be linked to nutrients sources.

- CHAPTER 7 -

Modelling microbial communities in Scottish sea lochs

7.1 Introduction

Mathematical models provide useful tools to investigate microbial food webs (Davidson 1996, Vallino 2000). These models cover a wide range of descriptive mechanism, from simple single organism models, such as the model of Monod for bacterial growth (1942), to the Cell quota models of Droop (1968) and Caperon and Meyer (1972), to simple interaction models such as the prey/predator model of Lokta-Voltera (1925).

The conceptual scheme developed by Steele (1974) summarised the basis of marine food chains: phytoplankton-zooplankton-fish. The microbial loop (Azam et al. 1983) extended this picture to include marine heterotrophic bacteria and nanoflagellates and its incorporation in marine ecosystems models led to their consequent improvement (Pomeroy 1974, LeB Williams 1981). Food web models are often formulated on a Nitrogen-Phytoplankton-Zooplankton-Detritus structure, such as the NPZD-model of Fasham et al. (1990). Other formulations of this NPZD model includes C to N stoichiometry (Taylor & Joint 1990), nutrient cycling (Moloney et al. 1986), and size-based interactions (Moloney & Field 1991). However, only a few models have been developed that include bacteria (Anderson & Williams 1998, Bissett et al. 1999, Vallino 2000).

The recognition of the microbial loop as a major feature of marine food chains highlights the importance of DOM cycling and its potential importance in models as a nutrient currency (C or N). However, “mathematical” cycling of DOM is poorly

understood (Kirchman 1993, Azam 1998). If the C:N ratio has been found to be relatively constant for phytoplankton and zooplankton, less for bacteria (see Chapter 4), this is not true for DOM C:N ratio which is highly variable. Therefore, in models that seek to adequately represent microbial communities, it is necessary to balance N cycling (N being the usual nutrient currency in models) with C. This has been undertaken in models by either simulating DOC alone, without associated DON, or simulating both quantities as separate independent state variables (Goldman & Dennett 2000, Christian et al. 2002). A difficulty of DOM modelling comes from the fact that numerous processes govern DOM production, e.g. phytoplankton exudation, grazer associated production, viral and bacterial lyses, solubilisation of particles and DOM removal, such as turn over of DOM, bacterial uptake vs. DOM lability, and photodegradation (Bjornsen 1988, Strom et al. 1997, Vetter et al. 1998, Bissett et al. 1999, Thingstad 2000). Although these processes are poorly understood and hence cannot be easily parameterised within models, some attempts of modelling DOM and bacterioplankton have given satisfactory simulations, such as Anderson et al. (1998), who simulated the seasonal cycle of DOC at the station E1 in the English Channel, or Polimene et al. (2006), who attempted to simulate complex interactions between DOM and bacteria.

In most modelling investigations of microbial food webs, the DOM pool that is available to uptake is assumed to be percentage of each pool and is based on DOC and DON concentration data (Anderson & Williams 1998). Due to the complexity of the DOM reservoir (due to lability and molecular weight) it is now recognised that simulating labile DOM, as a simple percentage of total DOM, does not allow proper characterisation of DOM cycling. The modelling study of Kelly-Gerreyn et al. (2004) is one of the few studies that discriminated labile, semi labile and refractory pools and the processes of formation and removal of each of these pools.

In fjordic sea lochs few attempts have been made to specifically simulate microbial processes using models. An exception is the model of Ross et al. (1993) developed for a range of locations (Killary Harbour, Loch Lihenne), including Loch Creran. Their model did not include the bacterial cycling of DOM, *per se*, but included DON that was excreted by phytoplankton and remineralised to inorganic N following a simple constant.

In this study, the existing Loch Creran model of Ross et al. (1993) was implemented and updated to include a fuller representation of microbial community dynamics. The main development in this work was the creation of a bacterial compartment in the model in order to investigate the potential to simulate bacterioplankton dynamics within such a model structure, the competition between phytoplankton and bacteria for inorganic resources and the associated dynamics of the fjordic microbial food web.

7.2 The model

7.2.1 Overview of the model

The model used in this study was developed by Ross et al. (1993) and revised by Ross et al. (1994). It is an ecosystem simulation for fjordic (e.g. restricted exchange) systems and its schematic structure is shown in figures 7.01 to 7.03, with equations and parameters defined in tables 7.01 to 7.05. This model was chosen because it was first developed for the study site of interest in this thesis (Loch Creran) and has been parameterised with data collected from this location (Ross et al. 1993). In this work, this model was complemented with a bacterial compartment to examine the effect of bacteria on the functioning of a marine food web in a fjordic system. As bacterial

growth is assumed to be based on organic carbon resources, a DOC compartment was also added.

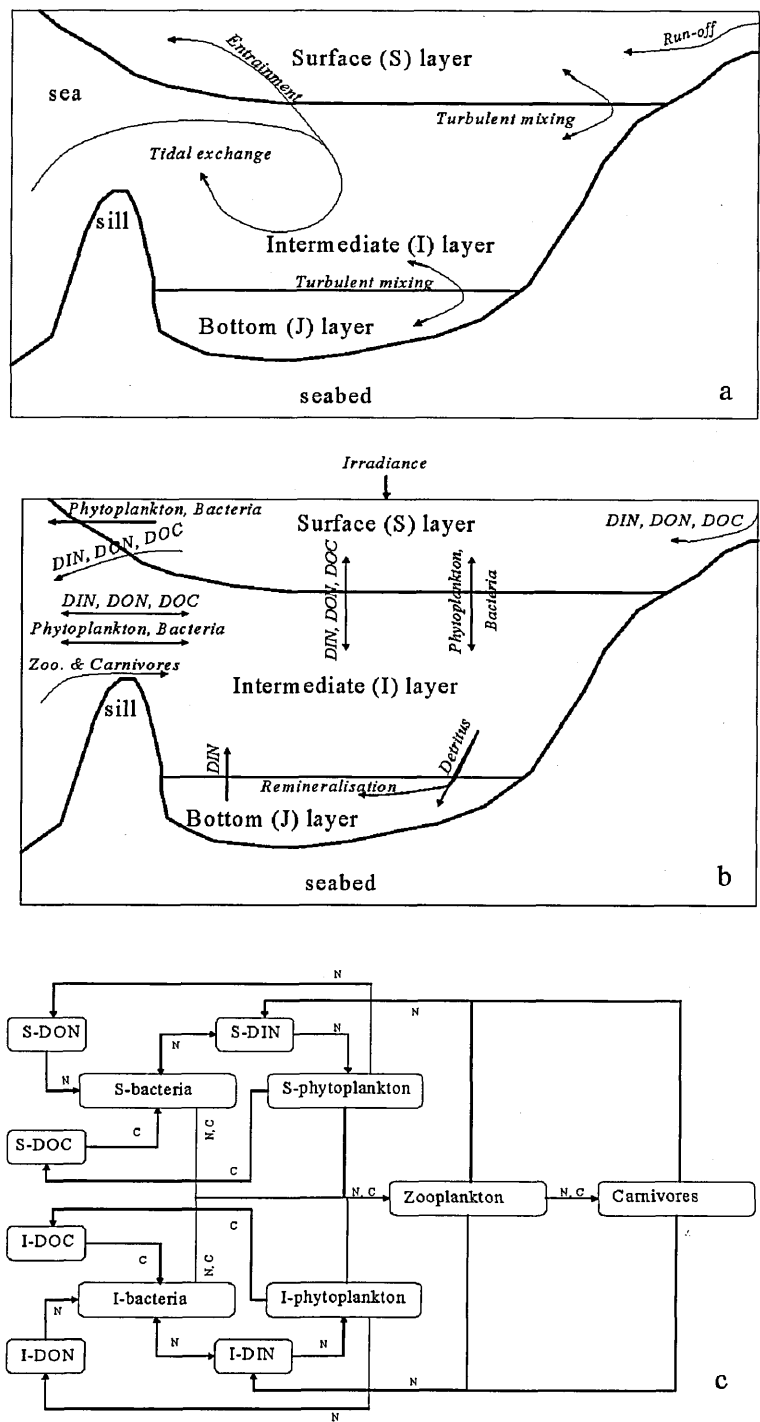


Figure 7.01. Diagrammatic representations of the physics (a), the biology (b) and the nutrient cycling (c) of the model use in this study.

7.2.2 The physical system

The vertical structure of the water column (Fig. 7.01) is separated into a surface (S) layer above the upper pycnocline, an intermediate (I) layer between the upper and lower pycnocline, and a bottom (J) layer between the lower pycnocline and the sediment. The physical modelling of these layers was simplified by assuming their respective thickness to be constant and that the three layers were well mixed vertically and horizontally.

The S and I layer are strongly coupled to the sea by tidal flushing (Fig. 7.01) and to each other by tidal upwelling (entrainment) and turbulent diffusion (simple diffusion law). The S layer received additional nutrient through runoff from land. Ross et al. (1993) demonstrated that the nutrient dynamics were close to that of a chemostat and were only weakly influenced by slow nutrient remineralisation through the sediment. Hence, these authors approximated the combined effects of the J layer and the sediment as a single nutrient compartment from which the nutrient is returned to the I layer by a first order rate process.

7.2.3 The biological system

The biota was originally composed of three functional groups: phytoplankton, zooplankton and carnivores (Fig. 7.02), to which a bacterial compartment in S and I layer was added in this study (see below). The phytoplankton of S and I layer were modelled separately but are coupled by sinking, upwelling and turbulent diffusion. Each phytoplankton assemblage takes up dissolved inorganic nitrogen (DIN) from its own layer and excretes dissolved organic nitrogen (DON) back into the same layer.

Table 7.01. State variables of the model from Ross et al. (1994), state variables developed in this thesis are highlighted in green in the table.

States variables	Units	Balance equations
Carnivore C concentration	mg C m^{-3}	$dC_C/dt = [a_C\Gamma - m_C C_C V_S + I_C(t)]/V_S$
Carnivore N concentration	mg N m^{-3}	$dC_N/dt = [a_C Q_Z \Gamma - m_C N_C V_S + Q_{IC}(t)]/V_S$
Zooplankton C concentration	mg C m^{-3}	$dC_Z/dt = [a_Z(G_{PS} + G_{PI} + G_{BS} + G_{BI}) - m_Z C_Z V_S - \Gamma + I_Z(t)]/V_S$
Zooplankton N concentration	mg N m^{-3}	$dN_Z/dt = [a_Z(Q_{PS}G_{PS} + G_{PI}Q_{PI} + Q_{BS}G_{BS} + Q_{BI}G_{BI}) - m_Z N_Z V_S - Q_Z\Gamma + Q_{IZ}(t)]/V_S$
Phytoplankton C concentration in S layer	mg C m^{-3}	$dC_{PS}/dt = [U_{CPS} - m_P C_P V_S - G_{PS} + M_{CIS} + W_{CRS} + W_{CIS}]/V_S$
Phytoplankton N concentration in S layer	mg N m^{-3}	$dN_{PS}/dt = [a_P U_{FPS} - m_P N_{PS} V_S - Q_{PS}G_{PS} + M_{NIS} + W_{NRS} + W_{NIS}]/V_S$
Phytoplankton C concentration in I layer	mg C m^{-3}	$dC_{PI}/dt = [U_{CPI} - m_P C_{PI} V_I + \delta_P C_{PS} V_S - G_I - M_{CIS} + W_{CEI}]/V_S$
Phytoplankton N concentration in I layer	mg N m^{-3}	$dN_{PI}/dt = [a_P U_{FPI} - m_P N_{PI} V_I + \delta_P N_{PS} V_S - Q_{PI}G_I - M_{NIS} + W_{NEI}]/V_S$
Bacteria C concentration in S layer	mg C m^{-3}	$dC_{BS}/dt = [U_{CBS}BGE - R_B - G_{BS} + W_{BCIS} + M_{CBIS} - \delta_B C_{BS} V_S]/V_S$
Bacteria N concentration in S layer	mg N m^{-3}	$dN_{BS}/dt = [U_{NBS} - X_{BS} - Q_{BS}G_{BS} + W_{NBIS} + M_{NBIS} - \delta_B N_{BS} V_S]/V_S$
Bacteria C concentration in I layer	mg C m^{-3}	$dC_{BI}/dt = [U_{CBI}BGE - R_B - G_{BI} + W_{BCEI} - M_{CBIS} - \delta_B C_{BI} V_I]/V_I$
Bacteria N concentration in I layer	mg N m^{-3}	$dN_{BI}/dt = [U_{NBI} - X_{BI} - Q_{BI}G_{BI} + W_{BNEI} - M_{NBIS} - \delta_B N_{BI} V_I]/V_I$
DIN concentration in J layer	mg N m^{-3}	$dF_J/dt = [\pi_d - k_{RF} F_J V_I]/V_I$
DIN concentration in I layer	mg N m^{-3}	$dF_I/dt = [X_{BI} + X_{CI} + X_{ZI} + k_{RF} F_J V_I - U_{FPI} - U_{FBI} + W_{FEI} - M_{FIS}]/V_I$
DIN concentration in S layer	mg N m^{-3}	$dF_S/dt = [X_{BS} + X_{CS} + X_{ZS} + k_{OR} D_S V_S - U_{FPS} - U_{FBS} + W_{FRS} + W_{FIS} + M_{FIS}]/V_S$
DON concentration in I layer	mg N m^{-3}	$dD_I/dt = [X_{PI} - k_{OR} D_I V_I - U_{NBI} + W_{DEI} - M_{DIS}]/V_I$
DON concentration in S layer	mg N m^{-3}	$dD_S/dt = [X_{PS} - k_{OR} D_S V_S - U_{NBS} + W_{DRS} + W_{DIS} + M_{DIS}]/V_S$
DOC concentration in I layer	mg C m^{-3}	$dO_I/dt = [e_{bp} C_{PI} - U_{CBI} - W_{OIS} + W_{OEI}]/V_I$
DOC concentration in S layer	mg C m^{-3}	$dO_S/dt = [e_{bp} C_{PS} - U_{CBS} + W_{OIS} + W_{OR}]/V_S$

Table 7.02. Physical quantities of the model from Ross et al. (1994), variables developed in this thesis are highlighted in green in the table.

	Description	Definition	Units
M_{CIS}	Net flux of C_P from I to S due to mixing	$T_{IS} (C_{PI} - C_{PS})$	$mg\ C\ d^{-1}$
M_{DIS}	Net flux of D from I to S due to mixing	$T_{IS} (D_I - D_S)$	$mg\ N\ d^{-1}$
M_{OIS}	Net flux of O from I to S due to mixing	$T_{IS} (O_I - O_S)$	$mg\ C\ d^{-1}$
M_{FIS}	Net flux from I to S due to mixing	$T_{IS} (F_I - F_S)$	$mg\ N\ d^{-1}$
M_{NIS}	Net flux of N_P from I to S due to mixing	$T_{IS} (N_{PI} - N_{PS})$	$mg\ N\ d^{-1}$
M_{BCIS}	Net flux of C_B from I to S due to mixing	$T_{IS} (C_{BI} - C_{BS})$	$mg\ C\ d^{-1}$
M_{BNIS}	Net flux of N_B from I to S due to mixing	$T_{IS} (N_{BI} - N_{BS})$	$mg\ N\ d^{-1}$
W_{CPEI}	Net flux of C_P from sea into I	$T_E [\Omega C_{PE}(t) - \beta C_{PI} - \Omega(1-\beta)C_{PI}]$	$mg\ C\ d^{-1}$
W_{CPIS}	Net flux of C_P from I to S due to upwelling	$\beta T_E (C_{PI} - \Omega C_{PS})$	$mg\ C\ d^{-1}$
W_{CRS}	Net flux of C_P from runoff into S	$T_R [C_{PR}(t) - \Omega C_{PS}]$	$mg\ C\ d^{-1}$
W_{DEI}	Net flux of D from sea into I	$T_E [D_E(t) - D_I]$	$mg\ N\ d^{-1}$
W_{DIS}	Net flux of D from I to S due to upwelling	$\beta T_E (D_I - D_S)$	$mg\ N\ d^{-1}$
W_{DRS}	Net flux of D from runoff into S	$T_R [D_R(t) - D_S]$	$mg\ N\ d^{-1}$
W_{OEI}	Net flux of O from sea into I	$T_E [e_{bP}C_{PE}(t) - O_I]$	$mg\ C\ d^{-1}$
W_{OIS}	Net flux of O from I to S due to upwelling	$\beta T_E (O_I - O_S)$	$mg\ C\ d^{-1}$
W_{OR}	Net flux of O from runoff	$T_R [O_R(t) - O_S]$	$mg\ C\ d^{-1}$
W_{FEI}	Net flux of F from sea into I	$T_E [F_E(t) - F_I]$	$mg\ N\ d^{-1}$
W_{FIS}	Net flux of F from I to S due to upwelling	$\beta T_E (F_I - F_S)$	$mg\ N\ d^{-1}$
W_{FRS}	Net flux of F from runoff into S	$T_R [F_R(t) - F_S]$	$mg\ N\ d^{-1}$
W_{CBEI}	Net flux of C_B from sea into I	$T_E [\Omega C_{BE}(t) - \beta C_{BI} - \Omega(1-\beta)C_{BI}]$	$mg\ C\ d^{-1}$
W_{CBIS}	Net flux of C_B from I to S due to upwelling	$\beta T_E (C_{BI} - \Omega C_{BS})$	$mg\ C\ d^{-1}$
W_{NBEI}	Net flux of N_B from sea into I	$T_E [\Omega N_{BE}(t) - \beta N_{BI} - \Omega(1-\beta)N_{BI}]$	$mg\ N\ d^{-1}$
W_{NBIS}	Net flux of N_B from I to S due to upwelling	$\beta T_E (N_{BI} - \Omega N_{BS})$	$mg\ N\ d^{-1}$
W_{NEI}	Net flux of N_P from sea into I	$T_E [\Omega N_{PE}(t) - \beta N_{PI} - \Omega(1-\beta)N_{PI}]$	$mg\ N\ d^{-1}$
W_{NIS}	Net flux of N_P from I to S due to upwelling	$\beta T_E (N_{PI} - \Omega N_{PS})$	$mg\ N\ d^{-1}$
W_{NRS}	Net flux of N_P from runoff into S	$T_R [N_{PR}(t) - \Omega N_{PS}]$	$mg\ N\ d^{-1}$

Phytoplankton sinking through the pycnocline is assumed to be dead and returns its N into the storage layer (J).

In the model, primary production (fixation of C by phytoplankton) was assumed to be limited either by irradiance or the availability of N. Nutrient limitation of phytoplankton growth (μ) is described using the cell quota model of Droop (1968) that postulates a minimum N level below which C fixation cannot take place and makes μ an hyperbolic function of the C:N ratio ("Q", cell quota). Light limitation (Andersen et al. 1987) and N uptake (Caperon & Meyer 1972) were approximated using Michaelis-Menten relationships. The cell quota equations were modified from Ross et al. (1994) and the uptake of N by phytoplankton was simulated as:

$$U_F = C_P \times S_P \times V_S \times \left(\frac{R_{MN} \times F}{H_{FP} + F} \right) \times \left(\frac{Q_{\max} - Q}{Q_{\max}} \right) \quad \text{equation 7.01}$$

where U_F is the phytoplankton uptake of N, C_P is the phytoplankton C concentration, S_P is the temperature dependence of phytoplankton rate processes, V_S is the volume of the S layer, R_{MN} is the maximum growth rate for N limited phytoplankton, F is the concentration of dissolved inorganic N (DIN), H_{FP} is the half-saturation phytoplankton DIN concentration, Q_{\max} is the maximum cell N quota and Q is the cell N quota.

This equation generates maximal uptake when the cell N quota (Q) is minimal and stops when cellular N tends or equals to maximal N quota (Q_{\max}) and is in accordance with the cell quota model of Droop (1968). The light limitation and attenuation of light equations were not modified from Ross et al. (1994), which takes in account the self-attenuation of light by phytoplankton (see Table 7.05, equations for γ_{SL} , $\kappa_{S,I}$ and U_{CP}).

Zooplankton feed on phytoplankton and bacteria from both S and I layer (Fig. 7.03) and excrete inorganic N into each layer in proportion to the ratio of time spend in each of the two layers.

Table 7.03. Physical parameters of the model from Ross et al. (1994).

	Description	Units	Value
V_B/V_S	Volume of bottom (J) layer / Volume of surface (S) layer	dimensionless	0.06
V_I/V_S	Volume of intermediate (I) layer / Volume of surface (S) layer	dimensionless	0.9
β	Proportion of tide upwelling into (S) layer	dimensionless	0.25
T_E/V_S	Volume exchange rate for tide into I layer / Volume of S layer	d^{-1}	0.37
T_{IS}/V_S	Volume exchange rate for mixing between I layer and S layer / Volume of S layer	d^{-1}	0.05
T_R/V_S	Volume exchange rate for runoff into S layer / Volume of S layer	d^{-1}	0.01
κ_0	Background attenuation coefficient	m^{-1}	0.22
γ_S	Mean depth of surface layer	m	8
γ_I	Mean depth of intermediate layer	m	8

Fecal pellets and corpses from zooplankton are assumed to sink rapidly through the lower pycnocline and release N directly into the storage layer. Carnivores are treated in the same way except that they feed exclusively on zooplankton. Prey:predator relationships were represented by Michaelis-Menten functions but with C (C concentration of the prey) as the limiting currency. Ingested C, and associated N, is assimilated with a fixed rate ($a_{Z,C,P,B}$, in Table 7.05) with unassimilated nutrient being rejected as fecal material or excreted as dissolved inorganic N.

Zooplankton and carnivores are capable of some mobility and therefore able to move between layers. This mobility especially affects the zooplankton grazing between S and I layers and also affects the layers receiving the waste products of this grazing activity. In the original version of the model, Ross et al. (1993) specified a fixed time spent by zooplankton in each layer (e.g independent of phytoplankton abundance) and

Table 7.04. Biological and nutrient parameters of the model, variables developed in this thesis are highlighted in green in the table. Parameters were taken from Ross et al. (1994) if not otherwise specified (see bottom of table).

	Description	Value	Units
Δ	Constant in function for zoo. egg production	100	d ⁻¹
Γ_{\max}	Max. carnivore predation rate	15	d ⁻¹
Ω	Phytoplankton and bacteria washout-retention factor	0.5	dimensionless
δ_C	Carnivore death rate	0.05	d ⁻¹
δ_P	Phytoplankton death rate	0.1	d ⁻¹
δ_Z	Zooplankton death rate	0.05	d ⁻¹
δ_B	Bacterial death rate	0.03	d ⁻¹
λ_C	Coefficient in carnivore temperature equation	1	dimensionless
λ_P	Coefficient in phytoplankton temperature equation	1	dimensionless
λ_Z	Coefficient in zooplankton temperature equation	1	dimensionless
λ_B	Coefficient in bacteria temperature equation	1	dimensionless
μ_C	Fraction of carnivore time spent in surface layer	0.5	dimensionless
θ_B	Bacterial C to N ratio	5.1	dimensionless
V_{\max}	Max. bacterial C, F uptake	6.9	d ⁻¹
ρ	Contribution of phytoplankton to attenuation coefficient	0.012	m ² (mg C) ⁻¹
τ_C	Coefficient in carnivore temperature equation	0.1	°C ⁻¹
τ_P	Coefficient in phytoplankton temperature equation	0.1	°C ⁻¹
τ_Z	Coefficient in zooplankton temperature equation	0.1	°C ⁻¹
τ_B	Coefficient in bacteria temperature equation	0.1	°C ⁻¹
ω_a	Constant in function for zoo. vertical migration	0.5	dimensionless
d_C	Fraction of carnivore uptake defecated	0.5	dimensionless
d_Z	Fraction of zoo. uptake defecated	0.36	dimensionless
eb_C	Fraction of carnivore biomass excreted per day	0.75	d ⁻¹
eb_Z	Fraction of zoo. biomass excreted per day	0.05	d ⁻¹
eb_P	Fraction of phytoplankton biomass excreted per day	0.34	d ⁻¹
eb_B ^a	Fraction of bacteria uptake excreted per day	0.15	dimensionless
BGE ^b	Bacterial growth efficiency	0.27	mg C (mg C) ⁻¹
e_{UC}	Fraction of carnivore uptake excreted	0.2	dimensionless
e_{UP}	Fraction of phytoplankton uptake excreted	0.05	dimensionless
e_{UZ}	Fraction of zoo. uptake excreted	0.15	dimensionless
$G_{P\max}$	Max. zooplankton grazing rate on phytoplankton	2	d ⁻¹
$G_{B\max}$	Max. zooplankton grazing rate on bacteria	0.5	d ⁻¹
H_{FP}	Half saturation phytoplankton DIN concentration	58.8	mg N m ⁻³
H_L	half saturation irradiance	60	μmoles m ⁻² s ⁻¹
H_C	half saturation phytoplankton C concentration	400	mg C m ⁻³
H_{ZP}	half saturation zooplankton C (phytoplankton) concentration	150	mg C m ⁻³
H_{ZB}	half saturation zooplankton C (bacteria) concentration	75	mg C m ⁻³
H_O ^a	Half saturation DOC concentration	13.4	mg C m ⁻³
H_{FB} ^a	Half saturation bacteria DIN concentration	7	mg N m ⁻³
k_{JR}	rate constant for sediment bottom remineralisation	0.01	d ⁻¹
k_{OR}	rate constant for DON remineralisation	0.02	d ⁻¹
Q_{IC}	N quota of immigrant carnivores	0.15	mg N (mg C) ⁻¹
Q_{IP}	N quota of immigrant phytoplankton	0.15	mg N (mg C) ⁻¹
Q_{IZ}	N quota of immigrant zooplankton	0.15	mg N (mg C) ⁻¹
Q_{\max}	Maximum N phytoplankton quota	0.25	mg N (mg C) ⁻¹
Q_{\min}	Minimum N phytoplankton quota	0.05	mg N (mg C) ⁻¹
Q_{off}	Storage switch transition width	0.01	mg N (mg C) ⁻¹
R_{ML}	Max. phytoplankton growth rate (light limited)	1.2	d ⁻¹
R_{MN}	Max. phytoplankton growth rate (N limited)	1.6	d ⁻¹
T_{ZE}	Constant in function for zoo. egg production	100	°C ⁻¹
U_{\max}	Max. phyto. N uptake rate per unit biomass	2.4	mg N (mg C) ⁻¹ d ⁻¹

^a from Anderson (1998); ^b from del Giorgio (1998)

assumed the uptake of C by zooplankton depended on phytoplankton C concentration. In a later version of the model (Ross et al. 1994) this behaviour was modified in response to phytoplankton density to model a “refuge effect”, in which the aggregations of zooplankton occurred in relation to high density of phytoplankton. In this PhD work, the original fixed fraction of time spent by zooplankton in each layer was preferred as a model of diel migration of zooplankton between I and S layers.

7.2.4 New additions to the model

The model was modified to include bacterial dynamics. Complementary to the bacterial component, a DOC compartment was created (Fig. 7.03). Similarly to DON, DOC is found in both S and I layer. DOC is exported into the I layer from the sea, then entrained or diffused into the S layer, which is also supplemented by DOC from runoff. DOC in both layers is also excreted by phytoplankton (Table 7.01). The dynamics of DOC were driven by bacterial uptake and physical exchanges (Table 7.02 and 7.04).

Bacteria were included in both the S and I layers (Fig. 7.01) responding to DOC stocks of both layers. The bacteria were made to take up DOC (U_{CB}) following the model of Monod (1942):

$$U_{CB} = \frac{V_{\max} \times O}{H_o + O} \quad \text{equation 7.02}$$

where O is the organic C concentration and the associated DON is taken up in stoichiometric proportion to C:

$$U_{NB} = U_{CB} \times Q_B \quad \text{equation 7.03}$$

with Q_B the N to C ratio of bacterial cell (see Table 7.05). The fixed remineralisation rate of DON to DIN of Ross et al. (1993) was therefore abandoned to the benefit of bacterial uptake of DON and excretion of N as a more realistic remineralisation pathway (see the model of Goldman et al. (1987), equation 4.01 in Chapter 4):

$$X_B = \left(\frac{1}{O/D} - BGE \times Q_B \right) \quad \text{equation 7.04}$$

where O is the organic C concentration and D , the organic N concentration (see Table 7.04 for the definition of the parameters in equations 7.02, 7.03 and 7.04). Similarly to phytoplankton, bacteria released inorganic N into the S and I layers and are grazed by zooplankton. Bacteria were also able to take up inorganic N according to a Michaelis-Menten relationship (see equation 7.02) but with a different affinity compared to DON (H_{FB} and H_{DB} in Table 7.05).

Zooplankton grazing was based on a Michaelis-Menten relationship and depends on bacterial C concentration but with different parameters to zooplankton grazing on phytoplankton giving a zooplankton feeding preference for the latter type of prey (Table 7.05). As for phytoplankton, this model simulated the transport of bacteria from the sea into the I layer, the upwelling of bacterial C to the S layer and the seaward flushing of bacteria from the S layer.

7.2.5 Driving function

The external environment of the system was modelled using driving functions (Fig. 7.02) for temperature, light irradiance, DIN, DON and DOC concentrations, and phytoplankton, immigration rates of zooplankton and carnivores.

Table 7.05. Biological quantities of the model from Ross et al. (1994), variables developed in this thesis are highlighted in green in the table.

	Description	Definition	Units
Γ	Total uptake of zoo. C by carnivores.	$C_C V_S \Gamma_{\max} S_C C_Z / (C_Z + H_Z)$	mg C d ⁻¹
γ_{IL}	Depth within I layer at which light becomes limiting	$\text{Min} \parallel \gamma_L, \langle \log_e \{ [L_S(t) \exp(-\kappa_S \gamma_S) / H_L] [(R_{ML} / \Psi_{IQ}) - 1] / \kappa_I \}^+ \parallel$	m
γ_{SL}	Depth within S layer at which light becomes limiting	$\text{Min} \parallel \gamma_S, \langle \log_e \{ [L_S(t) / H_L] [(R_{ML} / \Psi_{SQ}) - 1] / \kappa_S \}^+ \parallel$	m
$\kappa_{S, I}$	Attenuation coefficient of PAR in S and I layer	$\kappa_o + \rho C_{PS}, \kappa_o + \rho C_{PI}$	m ⁻¹
πD	Total production rate of corpses and fecal pellets	$d_C Q_Z \Gamma + d_Z (Q_{PS} G_S + Q_{PI} G_I) + \{ \delta_C N_C + [\delta_Z + \Delta \exp(-T_{ZE}/T)] N_Z \} V_S + \delta_P N_{PI} V_I$	mg N d ⁻¹
$\Psi_{SQ, SI}$	Depth average growth limitation of S and I phyto. by N	$R_{MQ} (1 - Q_{min}/Q_{PS})^+, R_{MQ} (1 - Q_{min}/Q_{PI})^+$	d ⁻¹
ac, Z, P	Fraction of carn., zoo., and phyto. uptake assimilated	$1 - d_C - e_{UC}, 1 - d_Z - e_{UZ}, 1 - e_{UP}$	dimensionless
G_S	Total uptake of S-layer phyto. C by zoo.	$\mu_Z C_Z V_S S_Z \langle \{ G_{Pmax} [C_{PS} / (C_{PS} + H_{ZP})] \} + \{ G_{Bmax} [C_{BS} / (C_{BS} + H_{ZB})] \} \rangle$	mg C d ⁻¹
G_I	Total uptake of S-layer phyto. C by zoo.	$(1 - \mu_Z) C_Z V_S S_Z \langle \{ G_{Pmax} [C_{PI} / (C_{PI} + H_{ZP})] \} + \{ G_{Bmax} [C_{BI} / (C_{BI} + H_{ZB})] \} \rangle$	mg C d ⁻¹
mC, Z, P, B	Fraction daily loss of carn., zoo., and phyto. biomass	$\delta_C + e_{bc} S_C, \delta_Z + e_{bz} S_Z + \Delta \exp(-T_{ZE}/T), \delta_P + e_{bp} S_P, \delta_B + e_{bB} S_P$	d ⁻¹
$Q_{PS, PI}$	Phytoplankton N quota in S and I layers	$[(1 - a_P) U_{FPS} / C_{PS} - U_{CPS} / C_{PS} Q_{PS}]$	mg N (mg C) ⁻¹
$Q_{BS, BI}$	Bacterial N quota in S and I layers	$N_{BS} / C_{BS}, N_{BI} / C_{BI}$	mg N (mg C) ⁻¹
Q_Z, C	Zooplankton and carnivore N quotas	$N_Z / C_Z, N_C / C_C$	dimensionless
Sc, Z, P, B	Temp. dependence of carn., zoo., phyto. and bacteria rate processes	$1 - \lambda \exp[-\tau_{CT} \theta(t)], 1 - \lambda \exp[-\tau_{ZT} \theta(t)], 1 - \lambda \exp[-\tau_{PT} \theta(t)],$ $1 - \lambda \exp[-\tau_{BT} \theta(t)]$	mg N (mg C) ⁻¹
U_{CPS}	Total uptake of C by S-layer phytoplankton	$C_{PS} V_S S_P \langle (\gamma_{SL} / \gamma_I) \Psi_{SQ} + (R_{ML} / \kappa_S \gamma_S) \log_e \{ [L_S(t) \exp(-\gamma_{SL} \kappa_S) + H_L] / [L_S(t) \exp(-\gamma_S \kappa_S) + H_L] \} \rangle$	mg C d ⁻¹
U_{CPI}	Total uptake of C by I-layer phytoplankton	$C_{PI} V_I S_P \langle (\gamma_{IL} / \gamma_I) \Psi_{IQ} + (R_{ML} / \kappa_I \gamma_I) \log_e \{ [L_S(t) \exp(-\gamma_{IL} \kappa_I - \gamma_{SL} \kappa_S) + H_L] / [L_S(t) \exp(-\gamma_{IL} \kappa_I - \gamma_{SL} \kappa_S) + H_L] \} \rangle$ $[V_{max} O_S / (H_O + O_S)], \quad \frac{1}{3} [V_{max} O_I / (H_O + O_I)]$	mg C d ⁻¹
$U_{CBS, I}$	Total uptake of C by S and I layer bacteria	$U_{CPS} Q_{BS}, U_{CBI} Q_{BI}$	mg C d ⁻¹
$U_{NBS, I}$	Total uptake of N by S and I layer bacteria	$C_{PS} V_S S_P \langle [U_{max} F_S / (F_S + H_{FP})] [(Q_{max} - Q) / Q_{max}] \rangle$	mg N d ⁻¹
U_{FPS}	Total uptake of N by S-layer phytoplankton	$C_{PI} V_I S_P \langle [U_{max} F_I / (F_I + H_{FP})] [(Q_{max} - Q) / Q_{max}] \rangle$	mg N d ⁻¹
U_{FPI}	Total uptake of N by I-layer phytoplankton	$V_{max} F_S / (H_{FB} + F_S), V_{max} F_I / (H_{FB} + F_I)$	mg N d ⁻¹
$U_{FBS, I}$	Total uptake of N by S and I layer bacteria	$\mu_C (e_{UC} Q_Z + e_{bC} S_C N_C V_S), (1 - \mu_C) (e_{UC} Q_Z + e_{bC} S_C N_C V_S)$	mg N d ⁻¹
$X_{CS, I}$	Total rate of excretion from carn. into S and I layers	$e_{UZ} G_S (Q_{PS} + Q_{BS}) + \mu_Z e_{bZ} S_Z N_Z V_S, e_{UZ} G_I (Q_{PI} + Q_{BI}) + (1 - \mu_Z) e_{bZ} S_Z N_Z V_S$	mg N d ⁻¹
$X_{ZS, I}$	Total rate of excretion from zoo. into S and I layers	$e_{UP} U_{FPS} + e_{bP} S_P N_{PS} V_S, e_{UP} U_{FPI} + e_{bP} S_P N_{PI} V_I$	mg N d ⁻¹
$X_{PS, I}$	Total rate of excretion from phyto. into S and I layers	$U_{CBS} \{ [1 / (O_S / D_S)] - (BGEQ_{BS}) \}, U_{CBI} \{ [1 / (O_I / D_I)] - (BGEQ_{BI}) \}$	mg N d ⁻¹
$X_{BS, I}$	Total rate of excretion from bact. into S and I layers		mg N d ⁻¹

Light irradiance data (Fig. 7.02-c) were modified from those of Ross et al. (1993, 1994) to use values collected from SAMS meteorological station during the study period. Light irradiance, sea temperature and DIN concentrations coming from the sea, runoff, zooplankton and carnivore immigration were approximated with sinusoidal functions following Ross et al. (1993, 1994).

Table 7.06. Driving functions of the model from Ross et al. (1994), functions used in this thesis are highlighted in green in the table.

	Descriptions	Units
$C_{PE}(t)$	Phytoplankton C concentration in the sea	mg C m^{-3}
$C_{BE}(t)$	Bacterial C concentration in the sea	mg C m^{-3}
$D_E(t)$	DON concentration in the sea	mg N m^{-3}
$D_R(t)$	DON concentration in runoff	mg N m^{-3}
$O_E(t)$	DOC concentration in the sea	mg C m^{-3}
$O_R(t)$	DOC concentration in runoff	mg C m^{-3}
$F_E(t)$	DIN concentration in the sea	mg N m^{-3}
$F_R(t)$	DIN concentration in runoff	mg N m^{-3}
$I_C(t)$	Total immigration rate of carnivores C	mg C d^{-1}
$I_Z(t)$	Total immigration rate of zooplankton C	mg C d^{-1}
$L_S(t)$	Irradiance in surface layer	$\mu\text{Einst m}^{-2} \text{s}^{-1}$
$N_{PE}(t)$	Phytoplankton N in the sea	mg N m^{-3}
$N_{BE}(t)$	Bacterial N in the sea	mg N m^{-3}
$\theta(t)$	Sea temperature	$^{\circ}\text{C}$

Phytoplankton C concentrations in the sea were estimated from chl *a* data collected at LY1 (see Chapter 3) following smoothing, using a three point moving average, and using a C to chl *a* ratio of one to 25.

DOC and DON concentration (Fig. 7.02-d and f) from the sea (LY1 station, Chapter 3) and from runoff measured for Loch Creran (see Chapter 3) were used to implement the driving functions of these quantities. It is worth noting here that DOC concentrations from runoff were higher than those in the sea (Fig. 7.02-d), making runoff the major contributor to DOC pool within the loch. However, DON concentration from runoff and the sea were comparable (Fig. 7.02-f). Similarly to phytoplankton, these data were smoothed using a three point moving average. A similar approach was used for bacteria where bacterial C from the sea was derived from

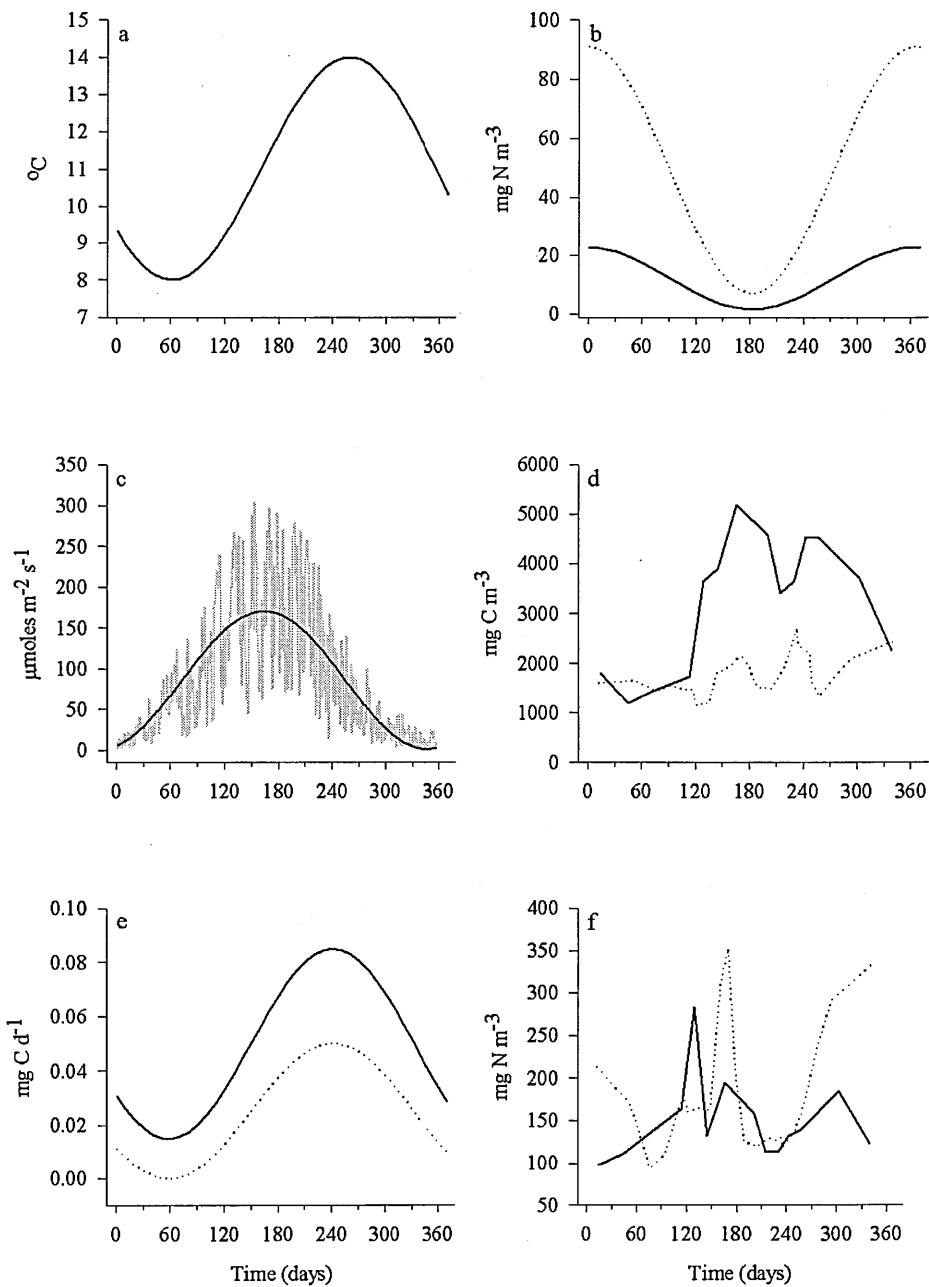


Figure 7.02. Main driving function used in the model with temperature (a), DIN from run (b-solid line) and from the sea (b-dotted line), light irradiance (c), DOC from runoff (d-solid line) and from the sea (d-dotted line), immigration of zooplankton (e-dotted line) and carnivores (e-solid line), and DON from runoff (f-solid line) and from the sea (f-dotted line).

bacterial abundance measurement at station LY1 (see Chapter 3), using the conversion factor of Fukuda (1998). Data for carnivores were kept as defined by Ross et al. (1993).

7.3 Implementation

The model was constructed with PowerSim (PowerSim Ltd, England), a mathematical simulation modelling software package. The set of differential equations was integrated with a 4th order Runge & Kutta algorithm, with variable time step, over a period of three cycles (3 years simulation). Simulations shown below are for the S layer as surface and intermediate layers demonstrated little difference. Observed data, against which the model was compared, were taken from station C5 in Loch Creran at 3 m depth (see Chapter 3).

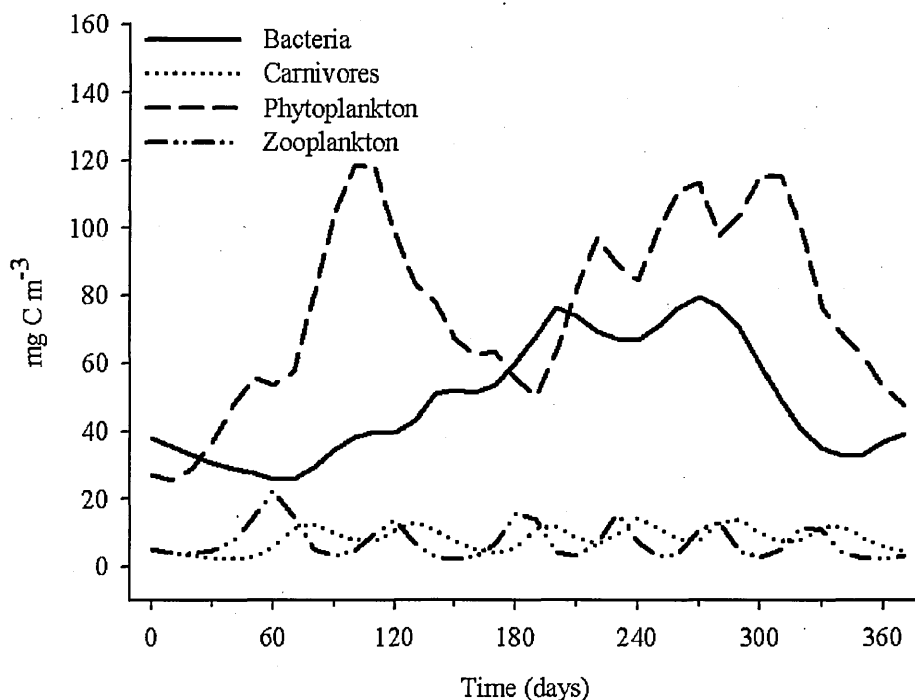


Figure 7.03. Annual cycle of bacteria (solid line), phytoplankton (dashed line), zooplankton (dash-dotted line) and carnivore (dotted line).

The initial aim of this work was to get an adequate representation of the annual cycle of the variables for Loch Creran, in order to validate the model simulations. The model was parameterised with data from Ross et al. (1993) and completed with data from Anderson et al. (1998) for bacterial uptake of DIN.

7.3.1 Standard run

The outputs of the standard run of the model are shown in figure 7.03. The seasonal variation of the phytoplankton shows two main peaks in spring and summer with low phytoplankton C concentration in the winter. The phytoplankton C concentration first increases to reach a spring maximum between days 90 - 120. A second gradual increase occurs in late summer autumn (days 240 - 330). Bacteria followed a different seasonal pattern (Fig. 7.03). The bacterial C concentration increased to a maximum around day 200, followed by another maximum around day 270. Bacterial C then decreased to lower levels in the winter time. Grazer, zooplankton and carnivore C concentration oscillated in response to prey growth. Zooplankton responds to phytoplankton and bacteria variations. Carnivores followed the pattern of zooplankton but with oscillations out of phase compared to those of zooplankton. These model outputs were then compared to the data collected from the field study (see Chapter 3). Comparisons between model output and data collected in Loch Creran are shown in figure 7.04.

The model predicted a peak in bacterial biomass in summer of similar magnitude to that observed. However, the observed peak of bacterial C occurred somewhat earlier in the year than the model prediction. The observation from station C5 also indicated one major peak of bacterial C occurring from the end of May into June. This peak is followed by a smaller one, around the third of the main peak, which

declines from September to reach its lowest values (during the winter). In comparison, the model predicted two peaks of bacterial C of similar magnitude within an annual cycle. These two peaks appear later in the annual cycle, which simulates bacterial C as remaining high at a season (from day 240) where it should be at its lowest level.

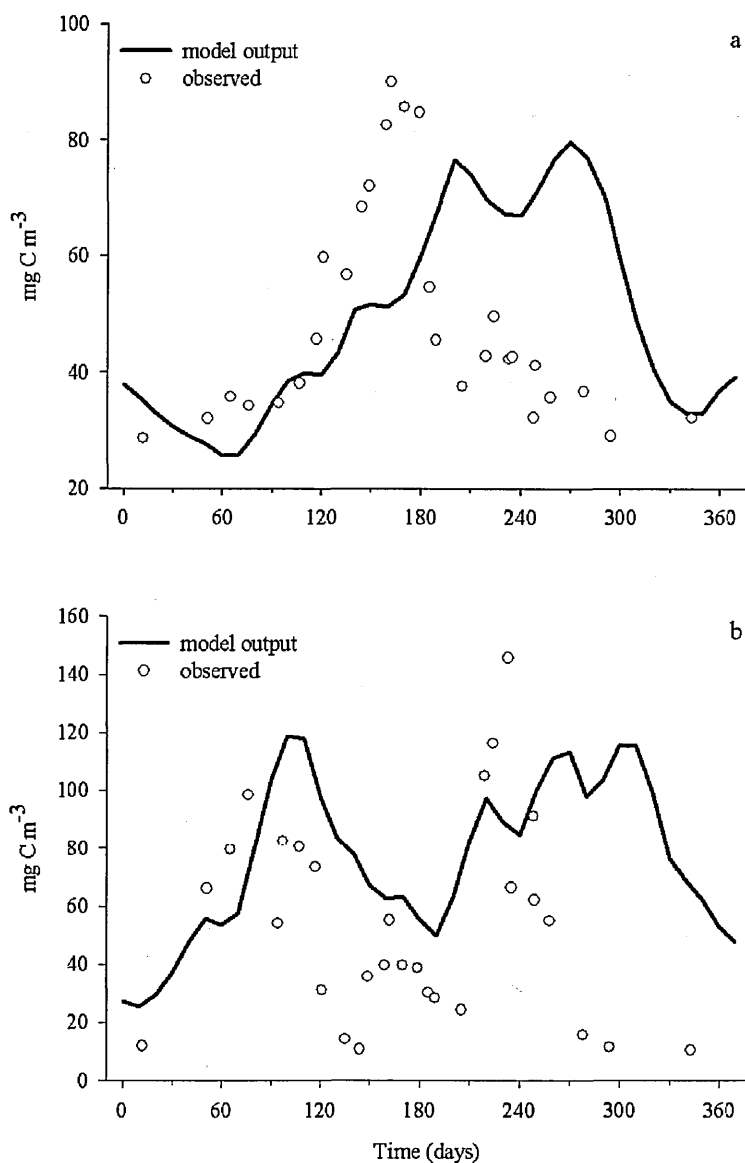


Figure 7.04. Comparison of model output (solid line) and data collected in Loch Creran (circle) for bacteria (a) and phytoplankton (b).

The annual integrated bacterial C of observed ($11.7 \text{ g C m}^{-3} \text{ y}^{-1}$) was lower, at about 54% ($6.3 \text{ g C m}^{-3} \text{ y}^{-1}$), than that predicted ($18 \text{ g C m}^{-3} \text{ y}^{-1}$) by the model.

Phytoplankton C concentration predicted by the model (Figure 7.04-b) gives a good fit of the observed seasonal variation of phytoplankton C. The model predicted the first spring increase, in terms of both timing and magnitude, as well as its subsequent decrease. The second increase of phytoplankton starts at the same time in the observed and predicted data, although, the model predictions slightly underestimated the values observed in Loch Creran. However, the model predicted the phytoplankton bloom to continue into the autumn, whereas the observations show it to decline in late summer. As with integrated bacterial C concentration, the annual integrated phytoplankton C concentrations were found lower than those predicted by the model (12.4 and 28.2 g C m⁻³ y⁻¹, for observed and predicted data respectively).

Dissolved inorganic (DIN) and organic (DOC, DON) nutrient data from the standard run are shown in figure 7.05. DIN concentrations predicted by the model present a good fit with the data collected at the C5 station, reproducing the main features (observed seasonal variations and range of values, Fig. 7.05-a). Concentrations decrease from day 1 to about day 120, remained close to 0 until day 300 and finally increase to high concentrations toward the end of the annual cycle. The predicted DON concentrations were in the range of values observed in Loch Creran. The predicted and observed seasonal variations of DON show two peaks (Fig. 7.05-b), the first of which was earlier than observed (around day 90 and 140, respectively); however, the second peak (day 330) predicted by the model was in agreement with the observations.

The predictions of the model always overestimated the concentrations of DOC compared to the observed values in Loch Creran (Fig. 7.05-c). However, the predicted and observed seasonal variations were similar but with the model output exhibiting greater amplitude. The DOC concentration values predicted by the model were close to those of the runoff formulated in the driving function. The model,

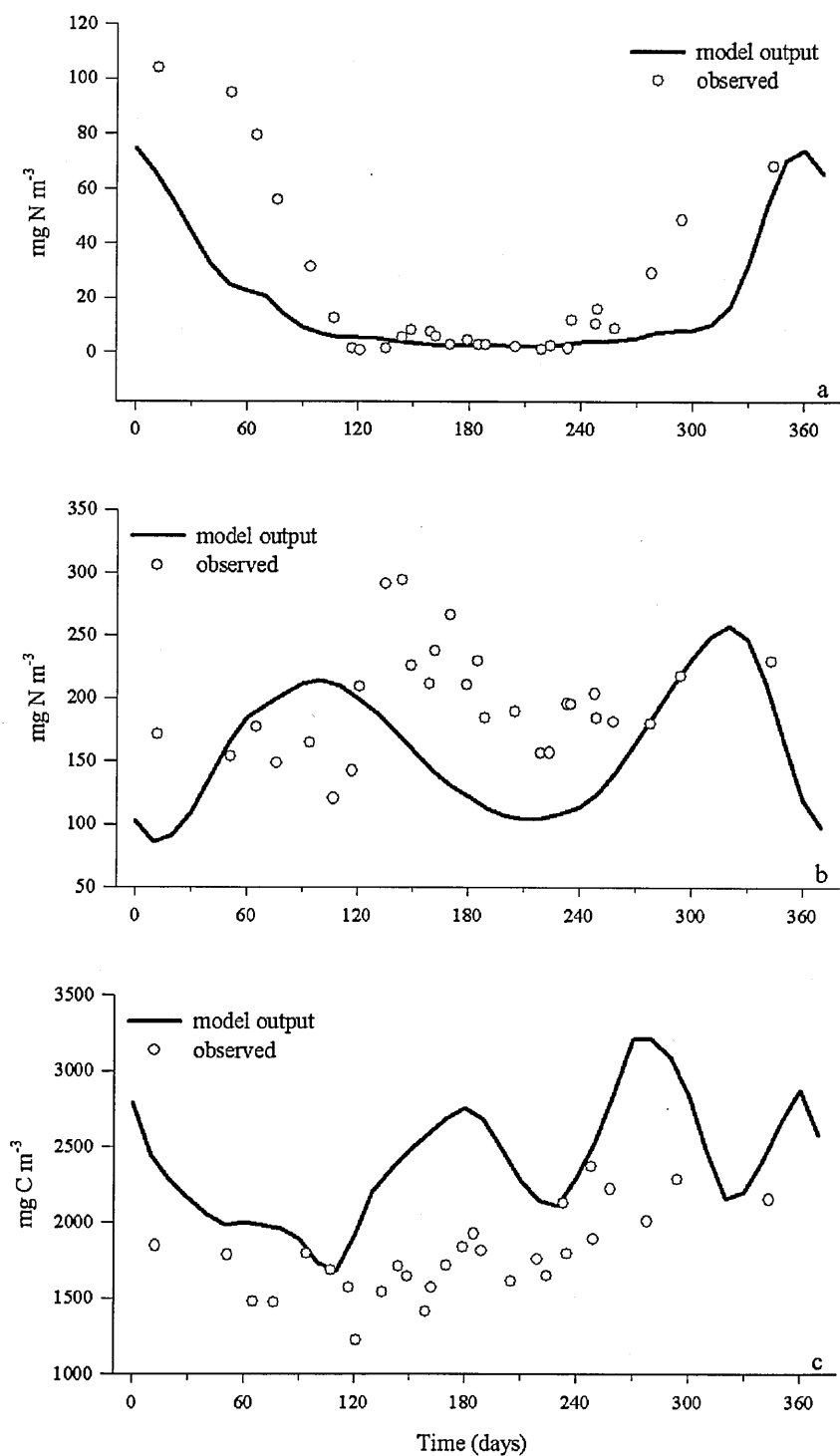


Figure 7.05. Comparison of model output (solid line) and data collected in Loch Creran (circle) for DIN (a), DON (b) and DOC (c).

therefore, satisfyingly predicted the major variations of nutrient concentrations and microbial dynamics observed in Loch Creran during this study. The simulated annual average bacterial and phytoplankton production were in the same range as observed values, although the model predicted slightly higher values. Seasonal cycles of phytoplankton, bacteria and nutrients were similar for observed and predicted data with occasional differences in timing.

7.3.2 Effect of grazing and DIN uptake by bacteria on the model behaviour

The model was used to investigate the role of bacteria in the fjordic ecosystem. In order to test the behaviour of the model to the addition of the bacterial compartment, two different scenarios were tested: (i) the standard run was compared with output obtained in the absence of grazers and (ii) the model was run with and without bacterial uptake of inorganic N (DIN). These two scenarios were investigated following observations made in Chapter 3, 5 and 6. In the first case, the model was used to support the hypothesis (presented in Chapter 3) that grazing controlled the bacterial population within the loch. In the second case the model was used to investigate the possible competition for inorganic nutrients between bacteria and phytoplankton as observed in Chapter 6.

The suppression of grazing on both phytoplankton and bacteria in the model provoked an increase of both phytoplankton and bacterial C concentration on an annual cycle (Fig. 7.06-a). In the absence of grazing, the integrated phytoplankton C concentration increased by 1.5 times compared to the value obtained during the standard run. Although the seasonal cycle was preserved, the peaks of phytoplankton C concentration were more prolonged which made the duration of elevated concentration

longer. In this case, phytoplankton C concentrations were above 100 mg C m^{-3} over a period of 230 days (two third of the year).

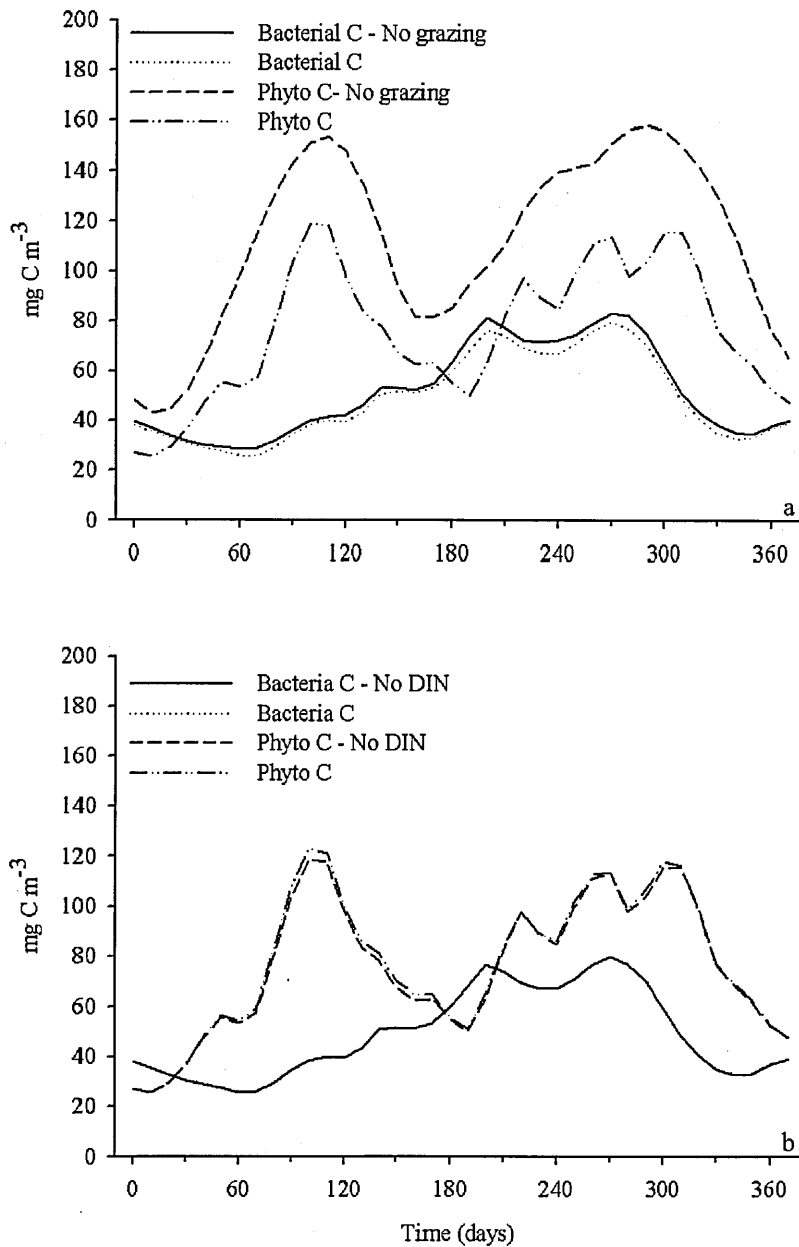


Figure 7.06. Scenario representing bacteria and phytoplankton (a) in absence of grazing (No grazing) and (b) in absence of DIN uptake by bacteria (No DIN).

Bacteria were less affected by the removal of grazing and less than a 6% difference in integrated values was observed between standard run and the run without

grazing pressure. As with phytoplankton, the absence of grazers did not affect the seasonal cycle of bacteria in the model. The bacteria were not affected by the cessation of DIN uptake and both the standard run and the run without DIN uptake were identical for bacterial biomass (Fig. 7.06-b). However, the modelled phytoplankton was affected when modelled bacteria stopped taking up DIN, with phytoplankton C concentrations found to be higher when bacteria did not compete for DIN. This difference was mainly noticeable for maximum phytoplankton C concentration and accounted for an increase in annual production of about 11% compared to the standard run.

7.4 Sensitivity analysis

The sensitivity analysis was conducted to investigate which parameter had the largest influence on the state variables in the model. Parameter sensitivity analysis adopted in this work was similar to that used by Fasham et al. (1990) where the output of a standard run was compared with the output of a run in which a single parameter was altered, first to a low value, then to a high value. The values of the parameter were, where possible, based on the knowledge of the likely range of variation; where this was not possible they were simply chosen to be half and twice the standard value. For each parameter tested, the maximal value of phytoplankton and bacterial C was recorded. For consistency, when maxima occurred on a different day relative to a standard run, the value for the standard day was taken into account (no matter if it was not the maximal value observed). The effect of the tested parameter, p , was quantified by calculating a normalised sensitivity, $S(p)$, defined as:

$$S_p = \frac{\left(\frac{E_p - E_s}{E_s} \right)}{\left(\frac{p - p_s}{p_s} \right)} \quad \text{equation 7.05}$$

where E_s is the value of maximal C (phytoplankton or bacteria) attained for the standard case with the parameter value p_s , and E_p is the value for the case when the parameter p was altered. This index measures the fractional change in the maximal C for a fractional change in the parameter. The higher, the index, the more sensitive the chosen output is to the variation of the parameter.

In total, 24 parameters (of 51 in the model) related to zooplankton, phytoplankton, bacteria and the physics of the model were tested in this sensitivity analysis (Fig. 7.07). The first output chosen was the maximum bacterial C concentration ($C_{b_{\max}}$) attained within an annual cycle. $C_{b_{\max}}$ was sensitive to bacterial growth efficiency (BGE) (Fig. 7.07-a), which defines the proportion of C uptake assimilated by bacteria (the higher, the more C is assimilated). The second important parameter affecting $C_{b_{\max}}$ was omega (Ω), which is related to the exchange-retention of bacteria (and phytoplankton) within the system. Surprisingly, $C_{b_{\max}}$ was less sensitive to DOC uptake parameters (V_{\max} , the maximal bacterial growth rate and H_O , the half saturation of DOC concentration) compared to BGE (Fig. 7.07-a).

The same set of parameters was also used to test the sensitivity of the model for the maximum phytoplankton C concentration ($C_{p_{\max}}$) reached in an annual cycle (Fig. 7.07-b). $C_{p_{\max}}$ was highly sensitive to Ω (see Table 7.04), particularly when the value of this parameter was halved. $C_{p_{\max}}$ was also sensitive to grazing related parameters such as $G_{p_{\max}}$, H_{ZP} (Table 7.04), but also related to excretion (eU_p) and maximum

growth rate (R_{ML} and R_{MQ} , Table 7.04). Finally, Cp_{max} was sensitive to variation of Beta (β), the proportion of tide upwelling into the S layer, which drives the entrainment of nutrients and organisms from the I layer to the S layer.

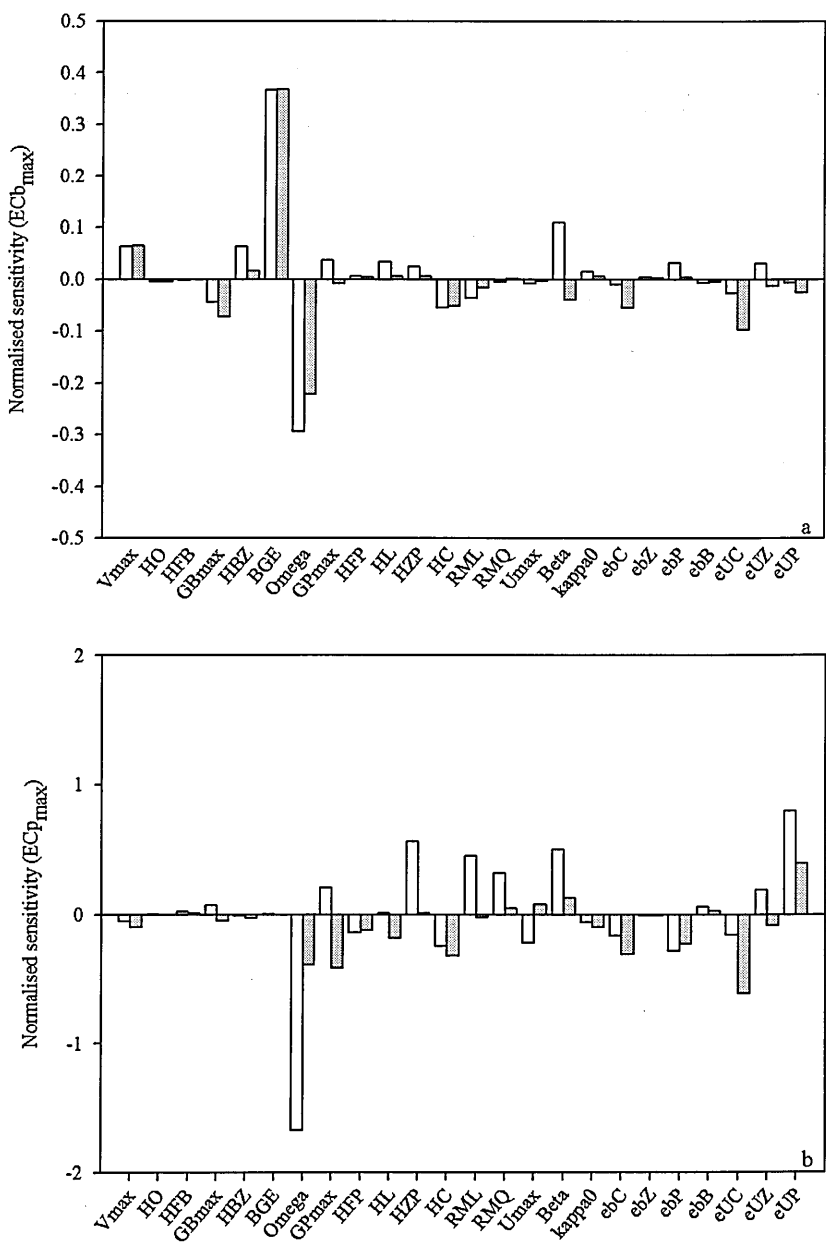


Figure 7.07. Normalized sensitivity carried out on 24 parameters (key for parameters is in Table 7.05) for Cb_{max} (a) and Cp_{max} (b).

7.5 Discussion

In this study, the model of Ross et al. (1994) was modified to incorporate a bacterial compartment and an associated DOC compartment. The modifications of the model aimed to more realistically simulate the DIN and DON cycling by bacteria. This modified model was then used to investigate the dynamics of organic nutrients (DOC and DON) within a fjordic system, in relation to the dynamics of bacteria, and to test the effect of grazing and competition for DIN between phytoplankton and bacteria, these considerations having been identified to be of potential importance by experimental work in Chapters 4, 5 and 6. The model contained 60 parameters and 14 driving functions for 19 state variables.

7.5.1 Driving functions

The driving functions were continuous representations of the best available data and were generally smoothed using a 3 points moving average (sea and runoff parameters) or approximated with sinusoidal or cosinusoidal functions (temperature, light irradiance and immigration curves).

The driving functions were modified from Ross et al. (1994). Light irradiance, for example, was corrected with data collected in 2005 from the SAMS meteorological station, these new data being higher than those used by Ross et al. (1994). The resulting higher light irradiance could potentially enhance the primary production in the present model, as Ross et al. (1994) concluded that primary production was light limited most of the year (according to their model). Phytoplankton from the sea was approximated from data collected at LY1 station in the Firth of Lorn and these data were preferred to those used in Ross et al. (1994), providing a more realistic input of phytoplankton and therefore C to the model.

7.5.2 Comparison of model output with observations from Loch Creran

An important point in the comparison of model output and data collected at station C5 station is that the later were transformed from either chl *a* concentration or cell numbers, using conversion factors. These conversion factors have a limited validity as they are not constant in nature and depend on many other conditions such as the species involved, the metabolic state of the cells, light and temperature (Geider 1987, Taylor et al. 1997). In this study, a C to chl *a* ratio of 1:25 was chosen, but the use of a 1:50 C to chl *a* ratio would have given closer results between modelled and observed annually integrated phytoplankton C concentration (2.6 and 2.8 g C m⁻³ y⁻¹ for observed and modelled data respectively).

Phytoplankton C concentrations calculated by the model were in agreement with the data collected at C5 station. However, the late summer early autumn bloom was of longer duration than the one observed. This means that modelled phytoplankton is still fixing C and taking up DIN whereas it should have naturally stopped. This suggests that phytoplankton benefit from other DIN sources to keep the production at such levels. DIN from the sea transported into the system (Fig. 7.02-b) is never exhausted and thus can be transported into the loch (by simple diffusion) to supplement the DIN stock available for phytoplankton uptake.

Bacterial increase started at the same time as the observations and increased to similar values, but did not decrease at the same time as the observations (Fig. 7.04-a). This suggested that there were sources of DOC in the model available to bacteria that potentially did not exist in the natural system. In fact, the DOC compartment in the model was considered as a single entity with no simulation of the different availability of compounds with regard to bacterial metabolism. This simplistic view was reflected in the equations used to model the C uptake by bacteria as data did not exist to parameterise variable lability of different fractions within the DOM pool. Therefore the

largest source of DOC (runoff in this model) would have the greatest influence on bacteria whereas, more realistically, some exudates from phytoplankton should be taken up preferentially (see Chapter 5) to DOC coming from runoff, much of which has potentially a terrigenous origin hence refractory character (see Chapter 3). It appears that modelled bacteria C concentrations follow the seasonality of runoff (Fig. 7.02-d) rather than phytoplankton, indicating that it was the source of organic C preferentially utilised by bacteria in the model. The availability of DOC from runoff could then be better parameterised by including a factor making available only a proportion of this DOC stock or including degradation steps via enzymatic reaction (Polimene et al. 2006). The uptake of DOC by bacteria is also subject to discussion as, for example, a constant V_{\max} and H_0 were used in the model and these are likely to vary with the season (Eichinger et al. 2006). This uptake may also vary with substrate C:N (see Chapter 4).

However, when the model was run with no DOC derived from run off (data not shown), the bacterial C concentration did not differ from that observed in the standard run. This suggested that the predicted prolonged bacterial C concentration peak was not due to DOC from run off entering Loch Creran and that other sources of DOC were responsible. The exchange of nutrients between the sea and the loch follow a simple diffusion law, including the rate of tidal exchange and the difference in concentration of the considered state variable between the sea and the loch; the concentration in the loch being weighted by a retention factor (Ω , fixed at 0.5, see Table 7.04). Consequently, nutrients were preferentially flushed into the loch and may have been responsible for the greater bacterial C concentrations predicted by the model.

7.5.3 "What if" scenarios

Although this model is a simplistic view of the microbial food web in Loch Creran, it is still a useful tool to compare or highlight some of the characteristics of this food web.

The model was developed with only one heterotrophic grazer (zooplankton) feeding on both phytoplankton and bacteria but with different maximum grazing rates and affinity; this enables differentiating prey preference. Although the existence of only one grazer is unrealistic, there is insufficient data available to justify the inclusion of a second grazer class in the model. Removing the grazing pressure on phytoplankton and bacteria in the model demonstrated the importance of grazing on the different prey dynamics. In the absence of grazing, phytoplankton C concentrations were enhanced by 1.5 times compared to the standard run. Furthermore, phytoplankton C concentration remained above a 100 mg C m^{-3} for over 230 days (two third of the year). With all other conditions remaining unchanged, it seems unlikely that nutrient and light in Loch Creran could sustain such high production predicted by this model at that time in the year.

The second point that was tested with this model is the potential competition for DIN between bacteria and phytoplankton. The model allowed bacteria to take up DIN (along with DON) following a simple hyperbolic function (Table 7.04) and hence to compete with phytoplankton for this resource. There were no differences in terms of bacterial C yield whether or not bacteria were taking up DIN (Fig. 7.06-b). This suggests that bacteria were not N-limited and, therefore, that the uptake of DOC was sufficiently complemented by DON to stoichiometrically balance the elemental (C and N) composition of the cells.

Modelled phytoplankton C concentration (C_P) was only slightly higher when bacteria were not competing for DIN. This is in agreement with the fact that, when

bacteria were not competing with phytoplankton, more DIN was available for phytoplankton growth promoting the further phytoplankton growth observed. It also suggests that bacterial competition with phytoplankton is insufficient to perturb the normal cycle of phytoplankton or its productivity.

7.6 Conclusion

The revised model presented in this Chapter satisfyingly reproduced the seasonal patterns for phytoplankton and bacteria observed at C5 station. However, it did lack accurate timing in the appearance of the blooms of phytoplankton, bacteria and the nutrient dynamics. As with Ross et al. (1993), one of the conclusions here is that the system was ultimately driven by the balance between import and export of nutrients. This simplistic model did not take in account all the complexity of the studied ecosystem. For example, the DOM pool was not differentiated into several DOC reservoirs reflecting different bacterial affinities. Nevertheless, the DOC and bacterial dynamics in this model highlight the non biologically available nature of the DOC derived from terrestrial runoff. Grazing control of bacteria was not demonstrated by the model, which does not agree with observations made in chapter 3, 4, 5 and 6. This may have been due to the fact that grazing on bacteria was not adequately represented and parameterised in the model.

- CHAPTER 8 -

General conclusion and future research work

8.1 Background

The recognition of the microbial loop triggered new research in the domain of marine microbiology. This link between DOM and higher trophic levels, that involves many different marine micro-organisms and microbial mediated processes, remains the subject of substantial ongoing research. The development of molecular tools, along with traditional ecological approaches, has made marine microbiology a subject rich in concepts, paradigm, but also paradoxes.

8.2 This study

This work consisted of three different approaches:

Field investigations (Chapter 3)

Laboratory experiments (Chapters 4, 5 and 6)

Modelling analysis (Chapter 7)

8.3 Major results

This study investigated the influence of organic nutrients (C and N) on microbial dynamics (bacterioplankton but also phytoplankton and nanoplankton). A comprehensive set of data was collected from three contrasting locations on the west coast of Scotland and was complemented with laboratory based experiments and modelling studies. This thesis therefore: i) provides information on the sources of

nutrients (inorganic and organic) affecting the locations studied, ii) examines the abundances of marine micro-organisms, their seasonal variations, their trophic relationships, and the ecological significance of the processes they mediate, iii) assesses the most likely factors driving the abundances and productivity of the studied ecosystems, and iv) investigates the different forms of organic nutrient perturbations likely to affect the microbial dynamics.

8.3.1 Sources of nutrients

Different sources of nutrients were investigated in this thesis. The concentrations of inorganic and organic nutrients in Loch Creran, River Creran and the Lynne of Lorn show seasonal variation (see Chapter 3), however, the relative influence or contribution of these different sources to the standing stocks measured at the three stations was more difficult to assess. If the fish farm does not seem to be a significant source of organic nutrients to Loch Creran (no statistical evidence of enhanced concentrations of DOC and DON in Loch Creran compared to LY1), especially compared to the influences of phytoplankton (see below), it was, however, a significant source of ammonium. This latter form of N, which is the most reduced, is known to be the preferred form of N to phyto- and bacterioplankton, however no direct evidence of increased biomass or production was found close to the FF station. Phytoplankton was found to be the major contributor to organic nutrient stocks in coastal waters (see Chapter 3, section 3.9.1.4), via the release of photosynthesis products (results from Chapter 5) but, due to the variety of sources, the fate of this DOM dynamics is not easily unravelled. However, the calculations presented in table 3.05 suggested a short turnover of the organic C compared to the accepted flushing time of Loch Creran suggesting an inadequate sampling strategy (see section 8.4 in this Chapter).

8.3.2 Organic nutrient perturbations

This work demonstrated that organic nutrients in coastal waters are of different characters, such as variable concentrations, stoichiometry, size fractions or bio-availability (Chapter 3, 4 and 5). Concentrations and stoichiometry of organic C and N appeared to govern bacterial metabolism (modification of bacterial C to N ratio, see Chapter 4). Furthermore, organic C:N stoichiometry may affect the C yield of a microbial system by creating limiting conditions (either C or N). However, C:N ratios do not seem to affect the growth efficiency of the system investigated (predator-prey) suggesting that the resources are optimally utilised (Chapter 4). Grazers efficiently removed bacterial production increasing C yield of the prey-predator system (Chapter 4) as more organic C was taken up. However, they seem rather inefficient in transferring N (regenerating the same amount of N as the bacteria alone) through the food chain and were found to preferentially regenerate N (Chapter 3 and 4).

Further investigations of the DOM produced by phytoplankton appeared to be affected by the N:Si ratio in terms of concentrations per unit of chl *a* (although chl *a* concentration may be affected by N availability, a DOC production relative to C per C would be preferred) and quality in terms of carbohydrates (Chapter 5). N:Si ratios are likely to vary in coastal areas; as shown in Chapter 3, and therefore may greatly influence the fresh DOM produced by the dominating phytoplankton. The availability of the DOM produced under inorganic nutrient stress plays a key role in controlling bacterial productivity and taxonomic composition (Chapter 5). This also suggests that bacteria were indirectly affected by inorganic nutrient disequilibrium, although inorganic nutrients are not thought to be the main bacterial energy resources (Chapter 3 and 5). Although inorganic nutrients may have an indirect effect on bacterial metabolism and taxonomy, bacteria are capable of directly competing with

phytoplankton for these nutrients. This hypothesis was tested using mathematical modelling and results revealed only a weak effect on phytoplankton production.

This work also revealed that, during the summer, the bacterial community seemed to be C limited, especially in Loch Creran. This C limitation would suggest that the DOM present at that time at LY1 and in Loch Creran was biologically non-available to bacteria or that bacterioplankton could be further limited by other conditions, such as trace metal.

8.3.3 Microbial dynamics

8.3.3.1 Phytoplankton

Typical phytoplankton seasonality was demonstrated with a diatom dominated spring bloom, followed by dinoflagellates and ciliates in the late summer and autumn. The effect of the seasons on phytoplankton community composition was clearly revealed by the MDS analysis (Chapter 3). Statistical analyses confirmed the relationships between environmental conditions and the occurrence of the different plankton groups. The diatom dominated spring bloom was thus related to nutrient concentrations and the summer bloom coincided with increasing water temperature. Phytoplankton and nanoplankton abundances suggested a potential inter-annual variability, with variation of the dominant diatom taxa from year to year during the spring, and the alternation of nanoflagellates with large dinoflagellates and ciliates in the summer. Phytoplanktonic community compositions also varied between LY1 and Loch Creran stations (Chapter 3), where different diatom species (*Skeletonema* at LY1 vs. *Chaetoceros* and *Thalassiosira* in Loch Creran) dominated the spring bloom at both stations. In the light of the specificity of phytoplankton DOM produced mentioned above, this would suggest a potential variability in DOM available to bacterioplankton.

8.3.3.2 Bacteria

In order to understand the dynamics of organic nutrients in Scottish coastal waters, an important part of this work as focused on bacterioplankton. Although there were no direct correlations between bacteria and DOM (C and N), the heterotrophic productivity was within the range of values previously reported in coastal environment (Chapter 3). The thymidine conversion factor was revealed to be a major source of variation in the calculations of bacterial production and should be more carefully considered in bacterial production studies (Chapter 3). These results demonstrated that between two stations (of about a mile apart of each other) BP at LY1 was overestimated when using literature based conversion factor. In contrast, BP was largely underestimated in Loch Creran using the same literature based conversion factor. Because BP at the fish farm was greater than LY1 when calculated using a concurrently derived TCF, it would suggest the presence of a more active bacterial community in Loch Creran (Chapter 3). The same conversion factor experiment could be carried out at C5 to test the applicability of these factors to the whole Loch Creran.

Although primary production was not directly assessed in this study, a tight coupling between primary production (using data obtained in earlier studies in Loch Creran) and bacterial production is possible (see Chapter 3, section 3.9.2.3) and may correlate with high production and fast turn-over of the organic matter mentioned above (Chapter 3 and 6). However, if this coupling is possible and has been observed in other coastal waters, bacterioplankton did not contribute to a constant fraction of the total particulate organic C, indicating the presence of effective removal processes (or control) of bacterial biomass and production (Chapter 3, 4,5 and 6). Bacteria were found to be controlled by heterotrophic nanoflagellates, but also by other heterotrophic grazers, as demonstrated by Gasol plots, which favoured the hypothesis of top-down control of the bacterioplankton. In additions, inter-annual variability in bacterial grazers was observed

between summer 2004, dominated by heterotrophic nanoflagellates, and the summer 2005, dominated by ciliates suggesting that HNAN were not solely responsible for this top-down control of bacterioplankton.

8.4 Future work recommended

This work demonstrated the necessity and the importance of considering the microbial community as a whole to characterise the dynamics of organic nutrient in temperate coastal waters. It also appears obvious that more work is required to better characterise, the size fraction and bioavailability of DOC and DON. This work could be complemented by including another important organic nutrient of ecological relevance, dissolved organic phosphorus: this work has detailed different problems encountered regarding to the DOM cycling in coastal waters. Consequently, more DOM ultrafiltration based experiments in conjunction with measurements of bio-availability (cell numbers or production) of the different fractions may help to better understand the characteristic of DOM in coastal waters.

The use of known tracers for example, either natural or synthetic, could be utilised in order to depict the different origins of dissolved organic nutrient in restricted-exchange systems and coastal waters: the use of stable isotopes or the characterisation of the lipid fraction (as a natural biomarker) of the DOM may give useful information on the origin (terrigenous or marine) of the DOM.

The field study and experimental work demonstrated the quick response of bacterioplankton, in term of biomass and production, sometime noticeable within 24 hours (see Chapter 5 and 6). This would further suggest that the sampling frequency should be adapted in studies investigating highly dynamic natural systems. In this work, the fortnight sampling regime may have resulted in incomplete understanding of the relationships between organic nutrients and microbial dynamics Scottish coastal waters.

Laboratory based experimental work confirmed that observation made in natural conditions and subsequent emerging hypothesis can be tested in controlled conditions. However, it is obvious that more laboratory work is required and this laboratory work could be supplemented with mesocosm experiments that would allow manipulation to be undertaken in “close to natural” conditions. This work has also highlighted the benefit of combining ecological approaches (production, grazing) with molecular biology, providing insights on the functionality of the different organisms involved. This is a key point to develop because our understanding of element (C, N and P) cycling in coastal waters and in the oceans is increasingly being recognised to relate to the functions fulfilled by the different organisms constituting the microbial food web.

This work could be reinforced by investigation of the broader marine microbial community including, for example, particle-attached bacteria. In addition, a better knowledge of microbial variables, such as grazing or size fractionated primary production, may help to improve our understanding of the role played by the different components of marine microbial communities. These parameters could be further used in modelling work. Finally, a refinement of the modelling approach is needed to better characterise the different sources (autochthonous v.s. allochthonous), compartments (available vs. refractory) and fractions (low and high molecular weight) of the dissolved organic matter pool, in relation to more fine scale microbial processes, such as enzymatic hydrolysis of large organic compounds.

8.5 Conclusion

This work draw attention to the necessity of including different scientific approaches in defining a frame work for the complex study microbial food web dynamics and dissolved organic matter pool. Azam (1998) emphasised the heterogeneity of time and space scales in his “plot thickens” and hypothesised that

“behavioural and metabolic responses of bacteria to the complex and heterogenous structure of the organic matter field at the micro-scale influence ocean basin-scale fluxes in all major pathways: microbial loop, sinking, grazing food chain, carbon storage, and carbon fixation itself”. The dynamics of the marine microbial communities observed at different scales of time and space (short time incubations to inter-annual variations or the contrast between coastal and fjordic systems) are an interesting point of the work detailed in this thesis. It demonstrates the importance of coastal biological systems, one of most productive, in the cycling of elements through very dynamic microbial food web. The concept of functional diversity (as opposed to specific diversity) that emerged from advances in genomic, phylogenetic or proteomic, gains increasing considerations in our understanding of marine microbial dynamics, although its use in ecological studies cannot be overstated (Pernthaler & Amann 2005). Therefore, the diversity, as species or function, has to be considered in the whole physico-chemical environment in relation with all the actors, competitors or predators, within the microbial food web.

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